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| <p>(54) Title: XANTHINE DERIVATIVES AS ADENOSINE A₁ RECEPTOR ANTAGONISTS</p> <p>(57) Abstract</p> <p>The present invention relates to a group of compounds which are xanthine derivatives and which act selectively at adenosine A₁ receptors. Adenosine antagonists acting at the A₁ receptor depolarize postsynaptic neurons and presynaptically enhance the release of a number of neurotransmitters, including acetylcholine, glutamate, serotonin, and norepinephrine. This action has potential for treating cognitive deficits such as those associated with Alzheimer's disease, as these transmitters have been implicated in learning and memory, and each of these transmitters is reduced in Alzheimer's disease. Some of the A₁ antagonist compounds described in the present invention increased the activity of hippocampal neurons in a long-term potentiation <i>in vitro</i> learning model, and antagonized a scopolamine-induced deficit in a water maze <i>in vivo</i> learning model. Thus, these compounds have potential for cognition-enhancing effects.</p> | | | |

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XANTHINE DERIVATIVES AS ADENOSINE A₁ RECEPTOR ANTAGONISTS**FIELD OF THE INVENTION**

The present invention relates to a group of compounds which are xanthine derivatives and which act selectively at 15 adenosine receptors. Adenosine antagonists acting at the A₁ receptor can depolarize postsynaptic neurons and can pre-synaptically enhance the release of a number of neurotransmitters, including acetylcholine, glutamate, serotonin, and norepinephrine, and thus have potential for treatment of 20 cognitive deficits and for cognition-enhancing effects.

BACKGROUND OF THE INVENTION

The profound hypotensive, sedative, antispasmodic, and vasodilatory actions of adenosine were first recognized 25 over 50 years ago. Subsequently, the number of biological roles proposed for adenosine have increased considerably. The adenosine receptors appear linked in many cells to adenylate cyclase. A variety of adenosine analogues have been introduced in recent years for the study of these 30 receptor functions. Alkylxanthines, such as caffeine and theophylline, are the best known nonselective antagonists of adenosine receptors.

Adenosine represents a general regulatory substance, 35 since no particular cell type or tissue appears uniquely responsible for its formation. In this regard, adenosine is unlike various endocrine hormones. Nor is there any evidence for storage and release of adenosine from nerve or

other cells. Thus, adenosine is unlike various neurotransmitter substances; it appears to be a neuromodulator rather than a neurotransmitter.

5

Although adenosine can affect a variety of physiological functions, particular attention has been directed over the years toward actions which might lead to clinical applications. It has now been recognized that there are 10 not one but at least two classes of extracellular receptors involved in the action of adenosine. One of these has a high affinity for adenosine and at least in some cells couples to adenylyl cyclase in an inhibitory manner. These have been termed the A₁ receptors. The other class of 15 receptors has a lower affinity for adenosine and in many cell types couples to adenylyl cyclase in a stimulatory manner. These have been termed the A₂ receptors.

The adenosine analogues exhibit differing rank orders 20 of potencies at A₁ and A₂ adenosine receptors, providing a simple method of categorizing a physiological response with respect to the nature of the adenosine receptor. The blockade of adenosine receptors (antagonism) provides another method of categorizing a response with respect to 25 the involvement of adenosine receptors.

Adenosine in the central nervous system (CNS) acts as a neuromodulator exerting its effects via the A₁ and A₂ receptors (G.L. Stiles, TIPS, 12, 486 (1986)). The 30 majority of adenosine receptors is localized in the brain, where A₂ receptors are found in the striatum, while A₁ receptors predominate in the hippocampus and in the cortex (areas involved in learning and memory). In general terms, A₁ receptors cause an inhibition of the release of 35 excitatory as well as of inhibitory neurotransmitters and a postsynaptic decrease of excitability. These effects are G-protein dependent, and they are mediated by an inhibition of adenylyl cyclase and of calcium influx and by an

-3-

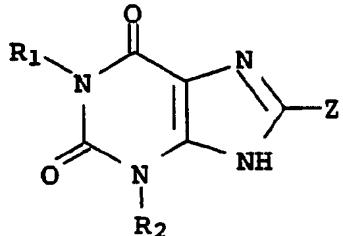
increase in potassium efflux (B.B. Fredholm, et al., TIPS, 9, 130 (1988)). In turn, A₁ antagonists can be expected to depolarize postsynaptic neurons and to presynaptically 5 enhance the release of various neurotransmitters (e.g. acetylcholine, glutamate, serotonin, and norepinephrine). This action could be of value in treating cognitive deficits such as those associated with Alzheimer's disease, as these transmitters have been implicated in learning and 10 memory, and each of these transmitters is reduced in Alzheimer's disease.

In view of the above, the compounds disclosed herein would be useful as agents for the treatment of cognitive 15 deficit disorders and conditions.

SUMMARY OF THE INVENTION

The present invention relates to compounds having the following general structures:

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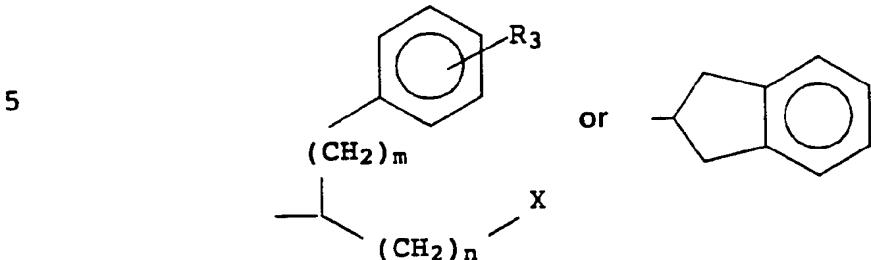


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including the (R) and (S) enantiomers and racemic mixtures thereof, and the pharmaceutically acceptable salts thereof, wherein R₁ and R₂ are each independently (C₁-C₄)lower alkyl or (C₂-C₄)lower alkenyl, Z is:

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-4-



- 10 R₃ is (C₁-C₃)lower alkyl, nitro, amino, hydroxy, fluoro, bromo or chloro, m is zero or an integer from 1 to 4, n is an integer from 1 to 4, and X is H or OH.

As used in this application the term (C₁-C₃)lower alkyl refers to methyl, ethyl, n-propyl, or isopropyl. Also, as used in this application the term (C₁-C₄)lower alkyl refers to methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl.

- 20 In addition as used in this application the term (C₂-C₄)lower alkenyl refers to ethynyl, propenyl, isopropenyl, butenyl, isobutenyl, etc.

Also, as used in this application, the substituent represented by R₃ may be at any position from 2-6 around the phenyl ring. There may be up to three such independent substitutions around the ring wherein the substituent is other than hydrogen.

- 30 Using a novel synthetic approach based on a unique binding mode proposed for xanthines at adenosine receptors, several 8-substituted xanthines have been synthesized. One of these compounds, 3,7-dihydro-8[(R)-1-methyl-2-phenyl-ethyl]1,3-dipropyl-1*H*-purine-2,6-dione is a potent, selective adenosine A₁ receptor antagonist based on displacement binding studies in rat brain (A₁ receptor Ki, 6.9 nM; A₂ receptor Ki, 157 nM). 3,7-dihydro-8[(R)-1-methyl-2-phenyl-ethyl]1,3-dipropyl-1*H*-purine-2,6-dione had little effect on

-5-

Type III or Type IV phosphodiesterase activity. In the anesthetized guinea pig, 3,7-dihydro-8-[(R)-methyl-2-phenylethyl]1,3-dipropyl-1H-purine-2,6-dione completely
5 reversed the A₁ receptor mediated decrease in heart rate produced by adenosine. In isolated guinea pig trachea, 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1H-purine-2,6-dione had very little effect on contraction produced by N⁶-cyclohexyl-adenosine, an A₁ selective
10 agonist. These observations suggest that multiple A₁ receptor subtypes may exist peripherally (Secrest, et al., FASEB Journal, 6, 1992).

We have now discovered, using the methods described in
15 more detail below, that the above compounds are useful as cognition enhancing agents and, therefore, that the compounds are useful in the treatment of conditions characterized by the exhibition of cognitive deficits.

20 3,7-Dihydro-8-[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1H-purine-2,6-dione and (R)-1,3-dipropyl-8-(1-phenyl-propyl)-xanthine [*aka* 3,7-dihydro-8-[(R)-1-phenylpropyl]1,3-dipropyl-1H-purine-2,6-dione] are potent and selective A₁ antagonists (Dudley et al., Soc. Neurosci. Abstr., 1992; Secrest, et
25 al.). These compounds have been tested in models of learning and memory both *in vitro* (hippocampal long-term potentiation) and *in vivo* (water maze learning).

Long-term potentiation (LTP) in the hippocampal slice
30 has been suggested as a model for events that might occur at a cellular level during learning and memory processes. (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1H-purine-2,6-dione increased the basal population spike
35 before and after induction of LTP recorded in hippocampal CA1 neurons. Similar results were found with another A₁ antagonist, KFM-19.

-6-

In the water maze spatial learning model, rats are required to use distal spatial cues around a circular water tank to navigate to a hidden platform. The cholinergic antagonist scopolamine dose-dependently impairs acquisition of the water maze task. (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine, 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione, and KFM-19 significantly antagonized the scopolamine-induced learning impairment.

10

These results (detailed below) indicate that the adenosine A₁ antagonists (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione have potential cognition-enhancing effects both *in vitro* and *in vivo*. (R)-1,3-Dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione, thus also have potential as a treatment for cognitive deficits.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1

Shows effect of (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine on scopolamine-induced impairment of water maze learning, showing the mean latency (\pm s.e.m.) to locate the platform over blocks of three trials of groups which receive vehicle plus vehicle (VEH-VEH), vehicle plus scopolamine at 1.2 mg/kg (VEH-SCOP 1.2), (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine at 5 mg/kg plus scopolamine at 1.2 mg/kg (MDL 5-SCOP 1.2), or (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine at 25 mg/kg plus scopolamine at 1.2 mg/kg (MDL 25-SCOP 1.2). † indicates significant difference from VEH-VEH group; * indicates significant difference from VEH-SCOP 1.2 group.

-7-

Figure 2

- Shows effect of 3,7-dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione on 5 scopolamine-induced impairment of water maze learning, showing the mean latency (ts.e.m.) to locate the platform over blocks of three trials of groups which receive vehicle plus vehicle (VEH-VEH), vehicle plus scopolamine at 1.5 mg/kg (VEH-SCOP 1.5), or 3,7-dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione at 25 mg/kg 10 plus scopolamine at 1.5 mg/kg (MDL 25-SCOP 1.5). "†" indicates significant difference from VEH-VEH group.

Figure 3a

- 15 Shows the percentage increase in the basal pop spike in rat brain slices versus time before and after induction of a "primed burst" following vehicle [DMSO] exposure.

Figure 3b

- 20 Shows the percentage increase in the basal pop spike in rat brain slices versus time before and after induction of a "primed burst" following drug [(R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine] exposure.

25 Figure 3c

- Shows the percentage increase in the basal pop spike in rat brain slices versus time before and after induction of a "primed burst" following drug [3,7-dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione]

30 exposure.

DETAILED DESCRIPTION OF THE INVENTION

RECEPTOR BINDING

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The following methods were used to examine representative compounds as disclosed herein for their A₁ and A₂ receptor-binding affinity.

Methods**A₁ Receptor Affinity**

5 The test described below was used to determine the potency of test compounds to compete with the ligand [³H]cyclohexyladenosine for the adenosine A₁ receptor prepared from rat brain membranes. Male Sprague-Dawley rats were sacrificed by decapitation and the membranes were
10 isolated from whole animal brains. See R. Goodman, et al., *Guanine Nucleotide and Cation Regulation of the Binding of [3H] Diethylphenylxanthine to Adenosine A-1 Receptors in Brain Membrane, Molecular Pharmacology*, 21, 329-335 (1982).

15 Membranes were homogenized (using polytron setting 7 for 10 seconds) in 25 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.7. The homogenate was centrifuged at 19,000 rpm for 10 minutes at 4°C. The pellet was washed by resuspending in 25 volumes of buffer with 2 IU of adenosine
20 deaminase per ml and incubated 30 minutes at 37°C. The homogenate was centrifuged again. The final pellet was resuspended in 25 volumes of ice-cold buffer.

25 The incubation tubes, in triplicate, received 100 µl of [³H]cyclohexyladenosine, 0.8 nM in the assay, 200 µl of test compounds at various concentrations over the range of 10-10 M to 10⁻⁶ M diluted with 50 nM Tris-HCl buffer (pH 7.7), 0.2 ml of membrane suspension (8 mg wet weight) and in a final volume of 2 ml with Tris buffer. Incubations were carried
30 out at 25°C for 2 hours and each one was terminated within 10 seconds by filtration through a GF/B glass fiber filter using a vacuum. The membranes on the filters were transferred to scintillation vials. The filters were counted by liquid scintillation spectrometry in 8 ml of Omniflour
35 containing 5% Protosol.

Specific binding of [³H]cyclohexyladenosine was measured as the excess over blanks taken in the presence of 10-

-9-

5 M 2-chloroadenosine. Total membrane-bound radioactivity was about 5% of that added to the test tubes. Specific binding to membranes was about 90% of the total bound.

- 5 Protein content of the membrane suspension was determined by the method of O.H. Lowry, et al., *Protein Measurements With Folin Phenol Reagent*, J. Biol. Chem., 193, 265-275 (1951).

Displacement of [³H]cyclohexyladenosine binding of 15%
10 or more by a test compound was indicative of affinity for
the adenosine binding site.

A₂ Receptor Affinity

The test described below was used to determine the potency of test compounds to compete with the ligand [³H]5'-N-ethyl-carboxamidoadenosine (NECA) for the adenosine A₂ receptors prepared from animal brain membranes. See R.R. Bruns, et al., *Characterization of the A-2 Adenosine Receptor Labeled by [³H]NECA in Rat Striatal Membranes*, Mol. Pharmacol., 29, 331-346 (1986). Young male rats (C-D strain), obtained from Charles River, were killed by decapitation and the brain was removed. Membranes for ligand binding were isolated from rat brain striatum. The tissue was homogenized in 20 vol ice-cold 50 mM Tris-HCl buffer (pH 7.7) using a polytron (setting for 6 to 20 seconds). The homogenate was centrifuged at 50,000 x g for 10 minutes at 4°C. The pellet was again homogenized in a polytron in 20 vol of buffer, and centrifuged as before. The pellet was finally resuspended in 40 vol of 50mM Tris-HCl (pH 7.7) per gram of 30 original wet weight of tissue.

Incubation tubes, in triplicate, received 100 µl of [³H]NECA (94 nM in the assay), 100 µl of 1 µM cyclohexyladenosine (CHA), 100 µl of 100 mM MgCl₂, 100 µl of 1 IU/ml 35 adenosine deaminase, 100 µl of test compounds at various concentrations over the range of 10⁻¹⁰ M to 10⁻⁴ M diluted with assay buffer (50 mM Tris-HCl, pH 7.7) and 0.2 µl of membrane suspension (5 mg wet weight), in a final volume of

-10-

1 ml of 50 mM Tris-HCl, pH 7.7. Incubations were carried out at 25°C for 60 minutes. Each tube was filtered through GF/B glass fiber filters using a vacuum. The filters were 5 rinsed two times with 5 ml of the ice-cold buffer. The membranes on the filters were transferred to scintillation vials to which 8 ml of Omnifluor with 5% Protosol was added. The filters were counted by liquid scintillation spectrometry.

10

Specific binding of [³H]NECA was measured as the excess over blanks run in the presence of 100 μM 2-chloroadenosine. Total membrane-bound radioactivity was about 2.5% of that added to the test tubes. Since this condition limits 15 total binding to less than 10% of the radioactivity, the concentration of free ligand does not change appreciably during the binding assay. Specific binding to membranes was about 50% of the total bound. Protein content of the membrane suspension was determined by the method of Lowry, 20 et al.

Displacement of [³H]NECA binding of 15% or more by a test compound was indicative of affinity for the adenosine A₂ site. The molar concentration of a compound which causes 25 50% inhibition of the binding of ligand was the IC₅₀. A value in the range of 100-1000 nM would indicate a highly potent compound.

Results

30 The following Table 1 shows the adenosine receptor binding affinities for several compounds.

TABLE 1
Adenosine Receptor Binding Affinity

| | <u>A₁ Ki</u> | <u>A₂ Ki</u> | <u>A₂/A₁</u> |
|--|-------------------------|-------------------------|------------------------------------|
| 35 3,7-Dihydro-8-[(S)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione | 60.7 nm | 848 nm | 14 |

-11-

TABLE 1
Adenosine Receptor Binding Affinity

| | | <u>A₁</u> Ki | <u>A₂</u> Ki | <u>A₂/A₁</u> |
|----|---|-------------------------|-------------------------|------------------------------------|
| 5 | 3,7-Dihydro-8-[(\pm)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione | 32.6 nm | 644 nm | 20 |
| | 3,7-Dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione | 6.9 nm | 157 nm | 23 |
| 10 | 3,7-Dihydro-8-[(S)-1-phenyl-ethyl]-1,3-dipropyl-1H-purine-2,6-dione | 174.7 nm | 12,900 nm | 74 |
| | 3,7-Dihydro-8-[(\pm)-1-phenyl-ethyl]-1,3-dipropyl-1H-purine-2,6-dione | 49.4 nm | 4,900 nm | 99 |
| 15 | 3,7-Dihydro-8-[(R)-1-phenyl-ethyl]-1,3-dipropyl-1H-purine-2,6-dione | 25.3 nm | 4,220 nm | 160 |
| | 3,7-Dihydro-8-[(\pm)-1-phenyl-methyl]propyl]-1,3-dipropyl-1H-purine-2,6-dione | 161.2 nm | 1,230 nm | 8 |
| 20 | 3,7-Dihydro-8-[(\pm)-1-(phenyl-methyl)butyl]-1,3-dipropyl-1H-purine-2,6-dione | 73.8 nm | 610 nm | 8 |
| | 3,7-Dihydro-8-[(\pm)-1-(2-indanyl)-1,3-dipropyl-1H-purine-2,6-dione | 64.3 nm | 8,350 nm | 129 |
| 25 | 3,7-Dihydro-8-[(\pm)-hydroxy-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione | 1,294 nm | 12,800 nm | 10 |
| | 3,7-Dihydro-8-[(\pm)-phenyl-propyl]-1,3-dipropyl-1H-purine-2,6-dione | 32.6 nm | 644 nm | 20 |
| 30 | 3,7-Dihydro-8-[(R)-phenyl-propyl]-1,3-dipropyl-1H-purine-2,6-dione {aka (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine} | 23.2 nm | 3,510 nm | 153 |
| | 3,7-Dihydro-8-[(S)-phenyl-propyl]-1,3-dipropyl-1H-purine-2,6-dione | 60.7 nm | 848 nm | 14 |
| 35 | 3,7-Dihydro-8-[(\pm)-2-phenyl-propyl]-1,3-dipropyl-1H-purine-2,6-dione | 94.3 nm | 1,740 nm | 18 |

-12-

TABLE 1
Adenosine Receptor Binding Affinity

| | | <u>A₁</u> Ki | <u>A₂</u> Ki | <u>A₂/A₁</u> |
|---|--|-------------------------|-------------------------|------------------------------------|
| 5 | 3,7-Dihydro-8-[(±)-trans-2-phenylcyclopentyl]-1,3-dipropyl-1H-purine-2,6-dione | 164.3 nm | 2,720 nm | 10 |
| | 3,7-Dihydro-8-[(±)-1,2,3,4-tetrahydro-2-naphthalenyl-1H-purine-2,6-dione | 94.6 nm | 10,300 nm | 108 |

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COGNITION-ENHANCING EFFECTS

The following methods were used to examine representative compounds as disclosed herein for their cognition-enhancing effects. The compounds were tested in animal models of learning and memory both *in vitro* (hippocampal long-term potentiation) and *in vivo* (water maze learning).

10 **Water Maze Learning Methods**

Training

Rats were trained in a 120-cm diameter water-filled tank to locate a hidden platform submerged just below the surface of the water. The location of the platform remained constant, but for each trial, the animal was required to swim from one of three different starting locations around the edge of the tank. There were no proximal cues in the tank, so the animal had to use a spatial mapping strategy using the distal cues around the room to navigate to the hidden platform. A computerized video tracking system allowed automated acquisition of the data. The animals were given 12 successive training trials during the single training day, using the methods of O. Buresova, et al., *Differential Effects Of Cholinergic Blockade On Performance Of Rats In The Water Tank Navigation Task And In A Radial Water Maze*, Behavioral Neuroscience, 100, 476-482 (1986). Each trial had a maximum duration of sixty seconds. If the animal did not locate the platform by that time, it was placed on the platform. After the animal found or was placed on the platform, it was allowed to stay there for 30 seconds. The next trial commenced immediately following the 30 second stay on the platform. Latency to locate the platform was recorded for each trial.

35 **Drug Administration**

Separate treatment groups of five animals each were run in each experiment. The VEH-VEH groups received vehicle (distilled water plus Tween) i.p. 40 minutes prior to the

-14-

first trial and vehicle i.p. 20 min. prior to the first trial. The VEH-SCOP groups received vehicle i.p. at 40 minutes and scopolamine HBr i.p. at 20 minutes. The MDL-
5 SCOP groups received (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine or 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione i.p. at 40 minutes and scopolamine HBr i.p. at 20 minutes.

10 Data analysis

The latency scores for each animal were averaged into four blocks of three trials each (one trial from each starting location). A one-way ANOVA comparing the treatment groups was computed on the scores for each trial 15 block. If the overall ANOVA was statistically significant, comparisons between individual treatment groups were made with Fisher's PLSD test.

Results And Discussion

20 (R)-1,3-Dipropyl-8-(1-Phenylpropyl)-Xanthine

The selective A₁ antagonist (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine reversed the scopolamine-induced impairment at 5 and 25 mg/kg i.p., as shown in Figure 1. The overall ANOVAs for blocks 2 and 3 were significant,
25 F(3,16)= 4.861, p<.02, and F (3,16)= 4.219, p=.02. Individual comparisons indicated that the VEH-SCOP group differed significantly from the VEH-VEH group (p<.05), and the MDL-SCOP groups did not differ significantly from the VEH-VEH group. In addition, the MDL 5-SCOP group differed from the
30 VEH-SCOP group on block 3 (p<.05), and the MDL 25-SCOP group differed from the VEH-SCOP group on blocks 2 and 3.

3,7-Dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione

35 The selective A₁ antagonist 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione partially reversed the scopolamine-induced impairment of water maze learning at 25 mg/kg i.p., as shown in Figure 2. The

-15-

overall ANOVAs for blocks 3 and 4 were significant, $F(2,13)=4.184$, $p<.05$, and $F(2,13)=4.881$, $p<.05$. Individual comparisons indicated that the VEH-SCOP group differed significantly from the VEH-VEH group ($p<.05$), and the MDL-SCOP group did not differ significantly from the VEH-VEH group.

These results indicate that (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione have potential cognition-enhancing effects and could be used to treat cognitive deficits.

We have obtained similar results in the water maze test with the selective A_1 antagonist KFM-19 (Boehringer Ingelheim), which has been reported to have activity in other animal models of learning and memory (G. Schingnitz, et al., *Selective A₁-Antagonists For Treatment Of Cognitive Deficits, Nucleosides & Nucleotides*, 10(5), 1067-76 (1991)).

Hippocampal Long-Term Potentiation Methods

In Vitro Hippocampal Slice

Slices were prepared from male Sprague-Dawley rats. Slice preparation techniques were modified from A.L. Mueller, et al., *Noradrenergic Responses In Rat Hippocampus: Evidence For Mediation By Alpha and Beta Receptors In The In Vitro Slice, Brain Res.*, 214, 113-26 (1981). Briefly, the hippocampus was rapidly and gently dissected over ice. 400 μ Sections were made with a MacIlwain tissue chopper and incubated on the interface of 34°C Kreb's buffer (124 mM NaCl; 4.9 mM KH₂PO₄; 2.4 mM MgSO₄; 2.5 mM CaCl₂; 25.6 mM NaHCO₃; 10 mM glucose) for at least one hour. Warm, moist 95% O₂/5% CO₂ was passed over the incubation chamber. To record, a slice was submerged in warmed, oxygenated media flowing at 2 ml/min.

Recording

A recording electrode was placed in the CA1 region of the hippocampus and a stimulating electrode was positioned 5 in the stratum radiatum of the CA3 region of this same slice. The bipolar stimulating electrode was made from a twisted pair of 62 μ nichrome wires. 100 μ sec pulses were applied at 30 second intervals and a glass recording electrode, 1-2 M Ω impedance, filled with 3 M NaCl was used 10 to carry the resultant CA1 population spike. The signal form the CA1 cell body layer was amplified, filtered (1 Hz- 4 KHz), and monitored on an oscilloscope. The evoked waveform was also digitized using a Computerscope A/D board (RC Electronics, Inc., Santa Barbara, CA) and stored for 15 analysis using Neuroscope software (Levin and Associates, Denver, CO) running on an IBM-AT. In order to insure the viability of the slice, "minimal viability criteria" were established. First, when the stimulus voltage was adjusted to evoke a threshold population spike the maximum EPSP had 20 to be greater than 1 mV. Also, the maximum population spike amplitude obtainable had to be greater than 5 mV. Finally, a paired pulse test was performed and a minimum paired pulse inhibition duration of at least 30 msec was established to insure the viability of inhibitory inter- 25 neurons (P. DiSenna, *Method And Myth In Maintaining Brain Slices*, in A. Schurr, et al., Eds. Brain Slices: Fundamentals, Applications, and Implications, (1987) Switzerland; S. Karger AG, 10-21). Only preparations that met these three criteria were used.

30

Twenty or more control population spikes were obtained to establish a baseline (at least 10 minutes). In most slices concentrated drug solutions were then diluted into the media for a 20 minute period to establish effects on 35 the baseline population spike. A "primed burst" tetanus was performed following the 20 minute drug exposure. One 100 μ sec pulse was followed by 4 similar pulses 170, 180,

190, 200 msec later. Drug superfusion was continuous throughout the experiment.

5 Drugs Used

Seven slices received DMSO, 0.25% (vehicle controls). Six slices were given (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine at 2.5 μ M and six slices were given 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione at 5 μ M. The compounds were dissolved in DMSO and diluted 1:400 into the perfusion media. In our hands, this dilution of DMSO had minimal effect on the slices.

Data Analysis

15 Each pop spike waveform was digitized, stored, and analyzed by the Neuroscope software. Pop spike amplitude was determined for each waveform and these amplitudes were normalized to pre-tetanus levels. Results were expressed as percent change from this control level and plotted vs. 20 time. Data from individual experiments were averaged across treatments using Lotus 123 and graphed.

Results And Discussion

3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-
25 1*H*-purine-2,6-dione increased the basal pop spike by 133%. (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine increased the basal pop spike by 130%. DMSO alone increased the basal spike by 7.8%. Following tetanus, the resultant LTP stabilized at 216% with 3,7-dihydro-8[(R)-1-methyl-2-
30 phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione, at 270% with (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and at 109% with DMSO alone. In addition, the LTP with 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione and (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine rose
35 sharply and remained stable throughout the observation period. LTP in tetanized control slices rose sharply but dropped to a stable level after 10-14 minutes (see Figure 3).

-18-

The results indicate that (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenyl-5 ethyl]1,3-dipropyl-1H-purine-2,6-dione have potential as treatments for cognitive deficits. The results we obtained are similar to those reported for another A₁ antagonist, KFM-19 (G. Schingnitz, et al.). (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine, 3,7-dihydro-8[(R)-1-methyl-2-10 phenylethyl]1,3-dipropyl-1H-purine-2,6-dione, and KFM-19 all enhanced synaptic transmission in the hippocampal slice. This enhancement of synaptic transmission is indicative of the cognition enhancing potential of a compound.

15

The present studies indicate that the adenosine A₁ antagonists (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine and 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1H-purine-2,6-dione have potential cognition-enhancing 20 effects both *in vitro* and *in vivo*. These compounds thus have potential as a treatment for cognitive deficits.

The following Table 2 summarizes some of the potential indications for the cognition-enhancing compounds disclosed 25 herein and illustrates some cognitive deficit improvement applications as well.

30

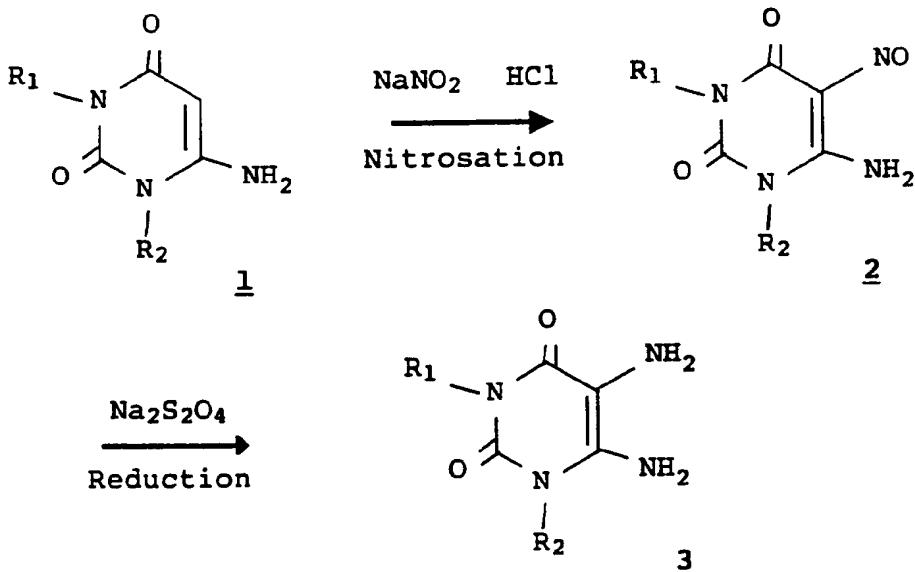
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-19-

TABLE 2POTENTIAL INDICATIONS FOR MEMORY-ENHANCING AGENTS¹

| | <u>Indication</u> | <u>Approximate Prevalence (1990)*</u> |
|---|--|---|
| <u>Deficit Improvement Applications</u> | | |
| 5 | Alzheimer's disease | 6.2 Million |
| 10 | Multi-infarct dementia | 4.2 Million |
| | Age-associated memory impairment (senility) | 60 Million |
| 15 | Korsakoff's syndrome | 22,000 |
| | Head-trauma-induced amnesia | 3.6 Million |
| | Other amnesias | 600,000 |
| 20 | Down's syndrome | 600,000 |
| | Attention deficit disorder | 5 Million |
| | Mental retardation | 5.5 Million |
| <u>Enhancement of Normal Function</u> | | |
| 25 | Adjunct to psychotherapy | 10-50 Million |
| | Scholastic performance | 20 Million |
| | Job performance | 270 Million |
| | Intelligence-gathering/ eyewitness accounts | 1-2 Million |
| 30 | * In the six major markets of France, Germany, Italy, Japan, the United Kingdom, and the United States | |
| | ¹ Cognos, Memory Enhancement, Decision Resource Inc., 17 New England Executive Park, Burlington, MA 01803 (June 1991) | |
| 35 | In general, compounds according to the invention can be made by following the procedures described in detail in Reaction Schemes I and II below. | |

-20-

REACTION SCHEME I

An appropriately alkyl substituted starting compound 1,
6-amino-2,4(1H,3H)-pyrimidinedione, wherein R₁ and R₂ are
defined as above, is chosen so that R₁ and R₂ are defined
20 the same as that desired in the final product.

The 6-amino-2,4(1H,3H)-pyrimidinedione is suspended in
water with 20% acetic acid. Sodium nitrite (1.5 equiva-
lents) in water is added in portions while keeping the
25 solution mildly acidic with concentrated hydrochloric acid.
The suspension is allowed to stir for several hours. It is
then filtered, rinsed with water and dried under vacuum to
yield the purple colored, alkyl substituted 6-amino-5-
nitroso-2,4(1H,3H)-pyrimidinedione (2).
30

The alkyl substituted 6-amino-5-nitroso-2,4(1H,3H)-
pyrimidinedione is then suspended in water, made alkaline
with 50% ammonium hydroxide (pH≈11) and treated with excess
35 sodium dithionite until the purple color fades. The reac-
tion is then extracted with chloroform. The organic
extracts are combined and dried over anhydrous magnesium
sulfate, filtered and concentrated under vacuum. The
residue is purified by flash chromatography (5% to 10%

-21-

methanol in chloroform). This material is then recrystallized from 10% isopropanol/hexane to yield the alkyl substituted 5,6-diamino-2,4-(1H,3H)-pyrimidinedione (3).

5 (See J.W. Daly, J. Med. Chem., 28, 487 (1985.)

10

15

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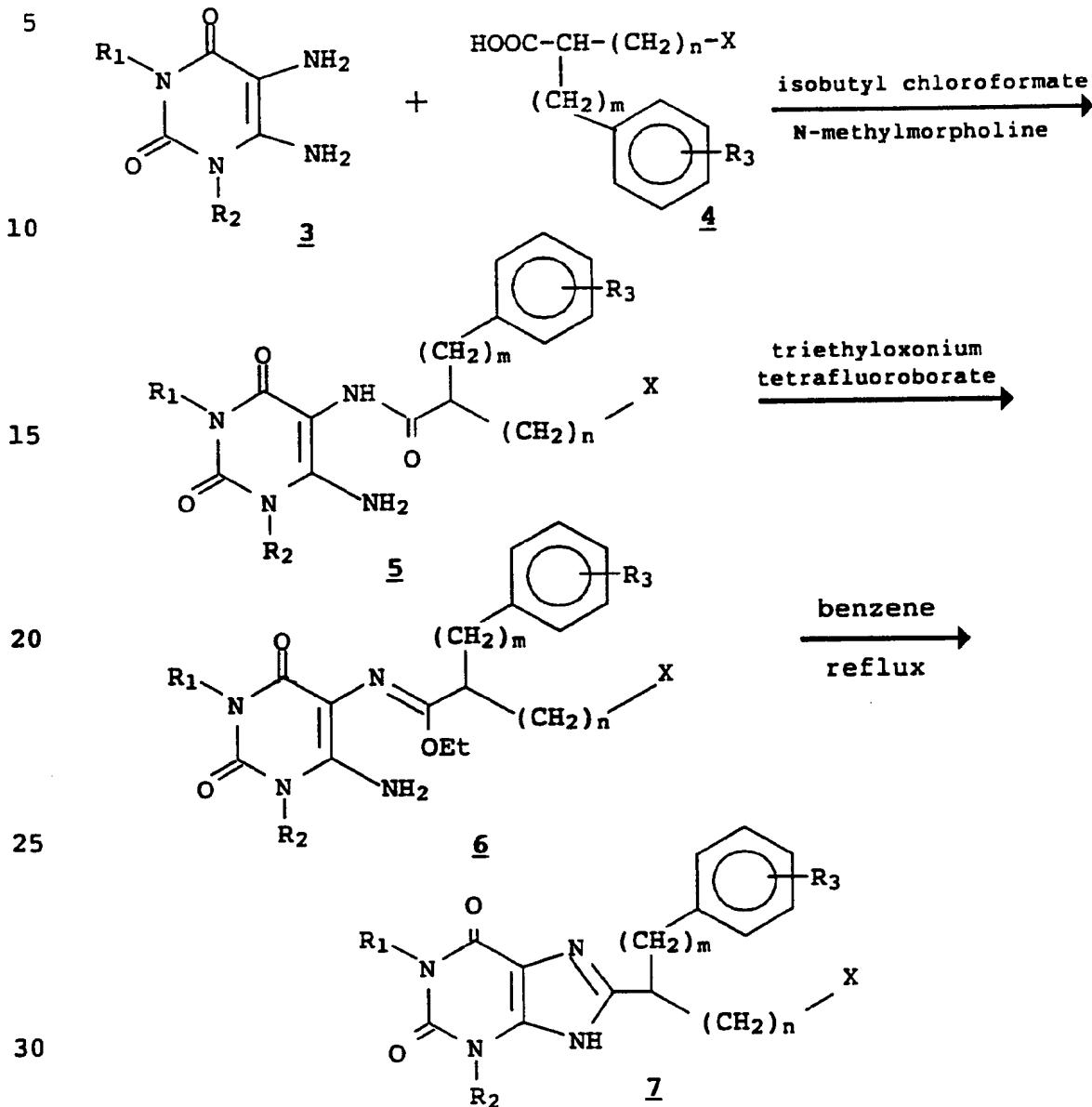
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35

Compound 3 from Scheme I is then reacted as shown in Scheme II.

REACTION SCHEME II



The alkyl substituted 5,6-diamino-2,4(1H,3H)-pyrimidine-dione (3) is then reacted with a 2-alkyl substituted alcanoic acid (4), wherein m, n, X and R₃ are defined as above. The acid (4) is chosen such that the definition of m and n are the same as that desired in the final product. It should be noted that the carbon atom designated by -CH- exhibits chirality and the acid should be chosen such that

-23-

the chirality is the same as that desired in the final product. Examples of such acids include the following:

- 5 S-(+)-2-phenylpropionic acid
 R-(-)-2-phenylpropionic acid

In addition, other such acids can be prepared as follows:



wherein m is an integer from 1 to 4, n is m-1 and X is a benzyl group of the structure



The acid is dissolved in tetrahydrofuran and treated with two equivalents of lithium diisopropylamide at room temperature. The reaction is heated to 40°C for 30 minutes. The reaction is then treated with one equivalent of benzyl chloride and heating is continued at 40°C for several hours. The reaction is then cooled to room temperature, poured into water and extracted with diethyl ether. The aqueous phase is then acidified with 1M hydrochloric acid and extracted with ether. The combined organic extracts are dried over anhydrous magnesium sulfate, filtered and concentrated. The residue is purified by radial chromatography (40% to 50% ethyl acetate in hexane, 2 mm plate) to yield the 2-alkyl substituted-3-phenylpropionic acid (4).

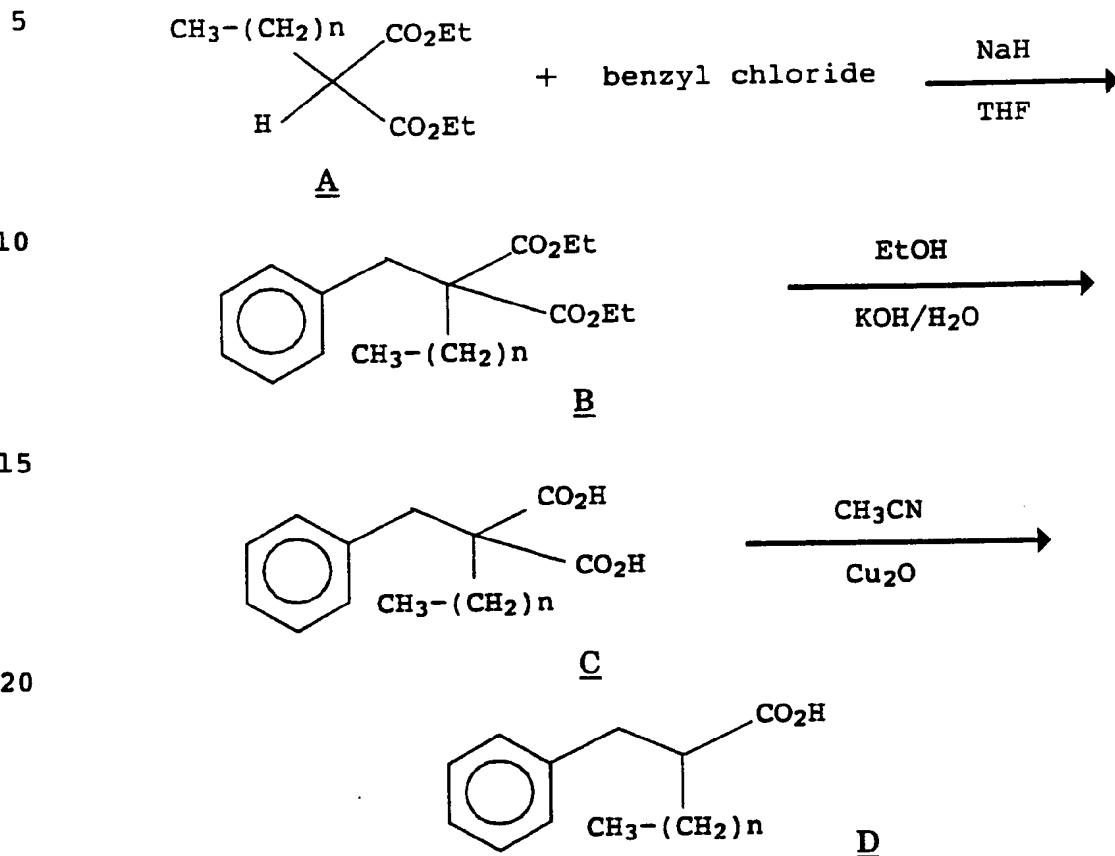
Examples of acids which can be reacted with benzyl chloride to form the 2-alkyl substituted-3-phenylpropionic acids are the following:

- 35 n-butyric acid
 n-valeric acid

-24-

In addition, other such acids can be prepared as shown in Scheme III.

REACTION SCHEME III



25

An appropriate alkyl substituted diethyl malonate (A), wherein n is defined as above is chosen such that n has the same definition as that desired in the final product. The malonate is added dropwise to a suspension of one equivalent of sodium hydride in tetrahydrofuran at 0°C. After stirring for approximately 30 minutes, one equivalent of benzyl chloride is added and the reaction is heated to reflux for approximately 3 hours. The reaction is then cooled, poured into water and extracted with ethyl acetate. The combined organic extracts are dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum to yield the alkyl substituted diethyl benzylmalonate (B).

The alkyl substituted diethyl benzylmalonate (B) is then treated with aqueous potassium hydroxide in ethanol and heated to reflux for 14 hours. After cooling, the 5 reaction is extracted with diethyl ether. The aqueous phase is then acidified with concentrated hydrochloric acid and extracted with diethyl ether. The combined organic extracts are dried over anhydrous magnesium sulfate and filtered and concentrated under vacuum to yield the alkyl 10 substituted benzylmalonic acid (C).

The alkyl substituted benzylmalonic acid (C) is then dissolved in acetonitrile and treated with a catalytic amount of cuprous oxide. (See M. Maumy, et al., Synthesis, 15 1029 (1986).) It is then heated to reflux for 5 hours. The solvent is then removed under vacuum. The residue is taken up in diethyl ether and rinsed with 10% hydrochloric acid followed by rinsing with saturated sodium chloride solution. The organic extract is dried over anhydrous 20 magnesium sulfate, filtered and concentrated under vacuum. The residue is purified by flash chromatography (5% to 10% methanol in chloroform) to yield the 2-alkyl substituted-3-phenylpropionic acid (4) (see Reaction Scheme II).

25 The 2-alkyl substituted-3-phenylpropionic acid (4) is then dissolved in tetrahydrofuran, treated with one equivalent of N-methylmorpholine (NMM) and cooled to -20°C. One equivalent of isobutyl chloroformate is added and the reaction is allowed to stir for approximately 30 minutes. 30 The alkyl substituted 5,6-diamino-1,3-dipropyluracil (3) in dimethylformamide is added and the reaction is stirred at -20°C for 4 hours. After warming to room temperature, the solvent is removed under vacuum. The residue is taken up in chloroform and rinsed with saturated sodium bicarbonate 35 solution, followed by rinsing with saturated sodium chloride solution. The organic extract is then dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The residue is purified by radial

-26-

chromatography (3% to 5% to 10% methanol in chloroform) (10% to 15% isopropanol in hexane) to yield amide (5).

5 The amide (5) is then dissolved in dry benzene and treated with 6.5 equivalents of triethyloxonium tetrafluoroborate (1M in dichloromethane). The reaction is heated to 50°C for approximately 2 hours. After cooling, the reaction is poured into phosphate buffer and extracted
10 with diethyl ether. The organic phase is rinsed with saturated sodium chloride solution, dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The residue is purified by radial chromatography (3% to 6% methanol in chloroform) to yield the imino ether (6).

15

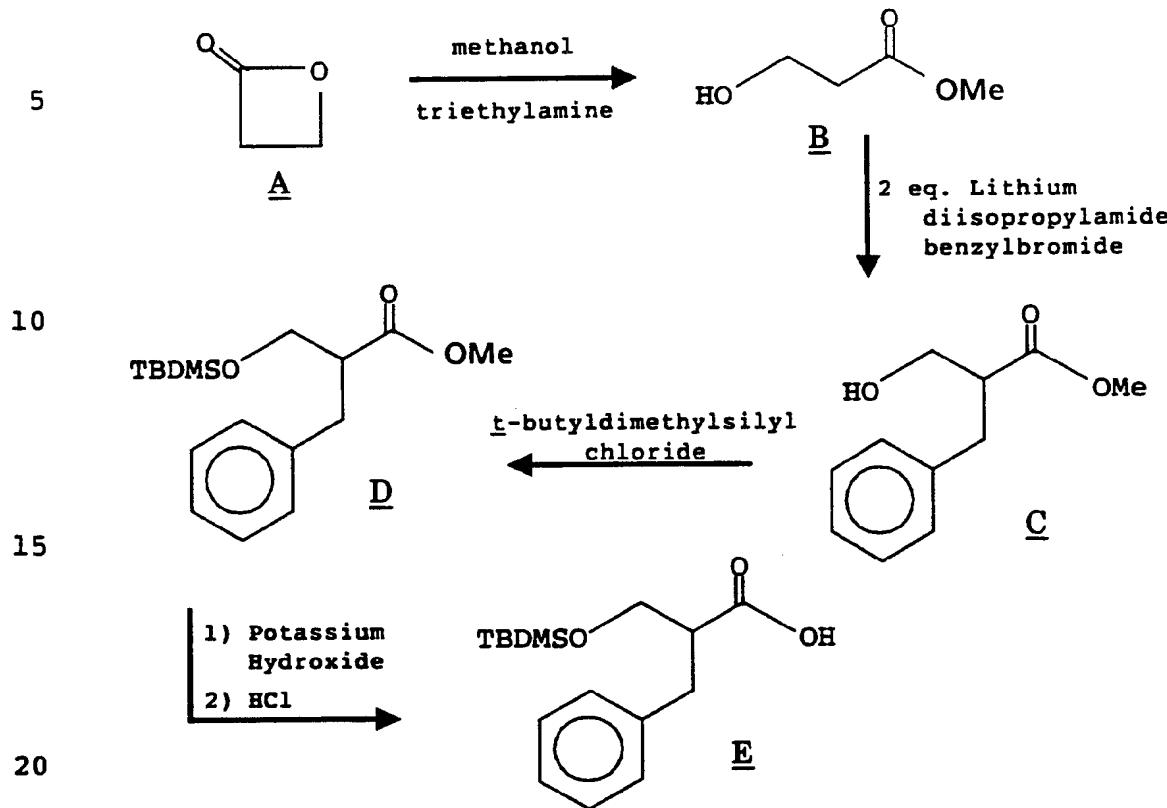
The imino ether (6) is then dissolved in dry benzene and heated to reflux for approximately 2 hours under nitrogen. The solvent is removed under vacuum and the residue is purified by radial chromatography (50% ethyl
20 acetate in hexane) to yield the 1,3-dialkyl-8-substituted xanthine (7).

25

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35

REACTION SCHEME IV



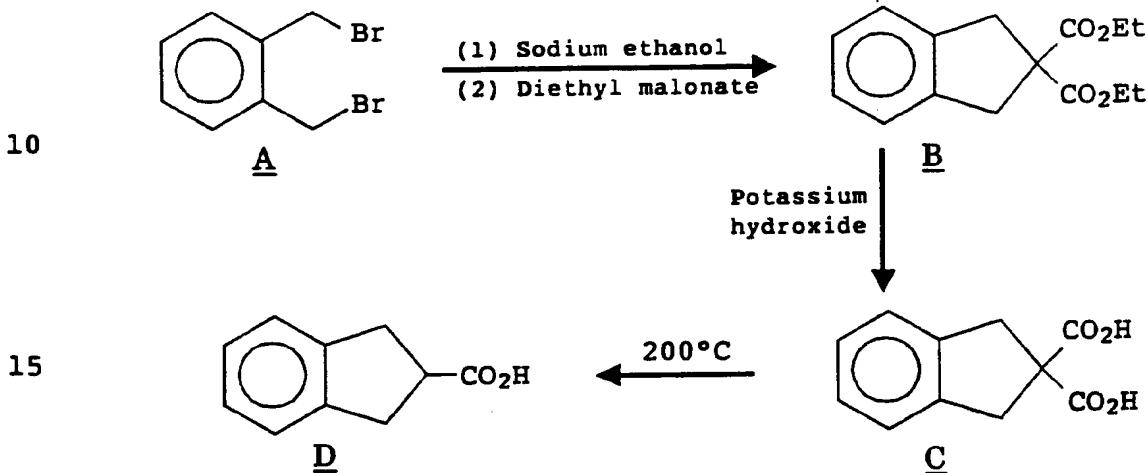
Another 2-subsituted alkanoic acid can be prepared as shown in Reaction Scheme IV above.

- 25 β -Propiolactone (A) is dissolved in methanol and
 treated with 1 equivalent of triethylamine to produce
 methyl 3-hydroxypropionate (B). Compound B is converted to
 the dianion with 2 equivalents of lithium diisopropylamide
 and alkylated with 1 equivalent of benzyl bromide to
 30 produce methyl 2-benzyl-3-hydroxypropionate (C). Compound
C is protected as the t-butyldimethylsilyl ether (D).
 Compound D is then saponified with potassium hydroxide and
 carefully acidified to produce the acid (E). The acid (E)
 is then dissolved in tetrahydrofuran, treated with 1 equi-
 35 valent of N-methylmorpholine and cooled to -20°. One
 equivalent of isobutyl chloroformate is added, followed by
 1 equivalent of 5,6-diamino-1,3-dipropyluracil in dimethyl-
 formamide to produce the amide. The amide is then treated

-28-

with aqueous potassium hydroxide at 70°C to produce the cyclized, deprotected 3,7-dihydro-8[(R)-1-methyl-2-phenyl-ethyl]1,3-dipropyl-1H-purine-2,6-dione.

5

REACTION SCHEME V

20 Another carboxylic acid which can be employed for the preparation of target compounds as illustrated in Reaction Scheme II can be prepared as shown in Reaction Scheme V above.

25 α,α -Dibromo-*o*-xylene (A) is treated with the anion of diethylmalonate at reflux to produce the diester (B). Compound B is then saponified with aqueous potassium hydroxide to produce compound C which is thermally decarboxylated at 200°C to produce the indan-2-carboxylic acid (D) (*J. Med. Chem.*, 32, 1989 (1989)). The acid (C) is then dissolved in tetrahydrofuran, treated with 1 equivalent of N-methylmorpholine, and cooled to -20°C. One equivalent of isobutyl chloroformate is added, followed by 1 equivalent of 5,6-diamino-1,3-dipropyluracil in dimethylformamide to produce 30 the amide. The amide is treated with aqueous potassium hydroxide and heated to reflux to produce the cyclized product, 3,7-dihydro-8-(2-indanyl)-1,3-dipropyl-1H-purine-2,6-dione.

35

-29-

The following list illustrates compounds according to the present invention:

- 5 3,7-Dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione
 3,7-Dihydro-8-[(S)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione
 3,7-Dihydro-8-[(R)-1-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione
10 3,7-Dihydro-8-[(S)-1-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione
 3,7-Dihydro-8-[1-(phenylmethyl)butyl]-1,3-dipropyl-1H-purine-2,6-dione
15 3,7-Dihydro-8-(1-phenylethyl)-1,3-di-2-propenyl-1H-purine-2,6-dione
 3,7-Dihydro-8-[1-(phenylmethyl)propyl]-1,3-dipropyl-1H-purine-2,6-dione
 3,7-Dihydro-8-(1-phenylethyl)-1,3-dipropyl-1H-purine-
20 2,6-dione.
 3,7-Dihydro-8-(1-phenylethyl-2-phenylethyl)-1,3-dipropyl-1H-purine-2,6-dione.
 3,7-Dihydro-8-(2-indanyl)-1,3-dipropyl-1H-purine-2,6-dione.
25 3,7-Dihydro-8-(hydroxymethyl-2-phenylethyl)-1,3-dipropyl-1H-purine-2,6-dione.
 3,7-Dihydro-8-[(R)-1-phenylpropyl]-1,3-dipropyl-1H-purine-2,6-dione.
 3,7-Dihydro-8-[(S)-1-phenylpropyl]-1,3-dipropyl-1H-purine-2,6-dione.
30

Pharmaceutical Preparations of the
Adenosine Receptor Agents

The preferred route of administration is oral administration. For oral administration the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, troches, lozenges, melts, powders, solutions, suspensions, or emulsions. The solid unit dosage forms can be a capsule which can be of the ordinary hard-5 or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, calcium phosphate, and cornstarch. In another embodiment the compounds of this invention can be tableted with conventional tablet bases such as lactose, sucrose, 10 and cornstarch in combination with binders such as acacia, cornstarch, or gelatin, disintegrating agents intended to assist the breakup and dissolution of the tablet following administration such as potato starch, alginic acid, corn starch, and guar gum, lubricants intended to improve the 15 flow of tablet granulations and to prevent the adhesion of tablet material to the surfaces of the tablet dies and punches, for example, talc, stearic acid, or magnesium, calcium, or zinc stearate, dyes, coloring agents, and flavoring agents intended to enhance the esthetic qualities 20 of the tablets and make them more acceptable to the patient. Suitable excipients for use in oral liquid dosage forms include diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a 25 pharmaceutically acceptably surfactant, suspending agent, or emulsifying agent.

The compounds of this invention may also be administered parenterally, that is, subcutaneously, intravenously, 30 intramuscularly, or interperitoneally, as injectable dosages of the compound in a physiologically acceptable diluent with a pharmaceutical carrier which can be a sterile liquid or mixture of liquids such as water, saline,

aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol, or hexadecyl alcohol, glycols such as propylene glycol or polyethylene glycol, glycerol
5 ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant such as a soap or a
10 detergent, suspending agent such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutically adjuvants. Illustrative of oils which can be used in the parenteral formulations of this invention
15 are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum, and mineral oil. Suitable fatty acids include oleic acid, stearic acid, and isostearic acid. Suitable fatty acid
20 esters are, for example, ethyl oleate and isopropyl myristate. Suitable soaps include fatty alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; anionic detergents,
25 for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates; nonionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepoly-propylene copolymers; and amphoteric detergents, for
30 example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, as well as mixtures. Alternatively, the compounds of this invention can be administered by aerosolization with a suitable carrier directly into the nasal passages, or by the administration
35 of droplets of a solution of the compounds of this invention, in an appropriate solvent, directly into the nasal passages.

The parenteral compositions of this invention will typically contain from about 0.5 to about 25% by weight of the active ingredient in solution. Preservatives and buffers may also be used advantageously. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a nonionic surfactant having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The exact amount of the compound or compounds to be employed, i.e., the amount of the subject compound or compounds sufficient to provide the desired effect, depends on various factors such as the compound employed; type of administration; the size, age and species of animal; the route, time and frequency of administration; and, the physiological effect desired. In particular cases, the amount to be administered can be ascertained by conventional range finding techniques.

The compounds are preferably administered in the form of a composition comprising the compound in admixture with a pharmaceutically acceptable carrier, i.e., a carrier which is chemically inert to the active compound and which has no detrimental side effects or toxicity under the conditions of use. Such compositions can contain from about 0.1 µg or less to 500 mg of the active compound per ml of carrier to about 99% by weight of the active compound in combination with a pharmaceutically-acceptable carrier.

The compounds may also be incorporated into any inert carrier so that they may be utilized in routine serum assays, blood levels, urine levels, etc., according to techniques well known in the art.

The compositions can be in solid forms, such as tablets, capsules, granulations, feed mixes, feed supplements and concentrates, powders, granules or the like; as well as liquid forms such as sterile injectable suspensions, orally administered suspensions or solutions. The pharmaceutically acceptable carriers can include excipients such as surface active dispersing agents, suspending agents, tableting binders, lubricants, flavors and colorants. Suitable excipients are disclosed, for example, in texts such as *Remington's Pharmaceutical Manufacturing*, 13 Ed., Mack Publishing Co., Easton, Pennsylvania (1965).

20 The following examples are presented to illustrate the present invention but they should not be construed as limiting in any way.

EXAMPLE 1

25 1,3-Di-n-propyl-6-aminouracil (30 g) was suspended in 1L of water with 41 ml of 20% acetic acid and overhead stirring. Sodium nitrite (9.03 g) in 41 ml of water was added in portions, keeping the solution acidic with 12 ml concentrated hydrochloric acid. A purple precipitate 30 formed. Addition was complete in 10 minutes and the suspension was allowed to stir for 2 hours. The solution was then filtered, and the filtrate was rinsed with water and dried under vacuum to yield 46 g of 1,3-di-n-propyl-5-nitroso-6-aminouracil.

35

The 1,3-di-n-propyl-5-nitroso-6-aminouracil (61.6 g) was suspended in 1L of water, and the suspension was made alkaline to pH 11 with 50% ammonium hydroxide and treated

with 100 g of sodium dithionite, in portions, until the purple color faded. The aqueous mixture was extracted with chloroform (8 x 200 ml), dried over magnesium sulfate,

5 filtered and concentrated. The residue was purified by flash chromatography (5/10% methanol/chloroform) and recrystallized from 10% isopropanol in hexane and recrystallized from 10% isopropanol to yield 37.29 g of 1,3-di-n-propyl-5,6-diaminouracil, m.p., 127-128°C.

10

Sodium hydride (50% suspension in mineral oil, 15.2 g) was rinsed with 100 ml of tetrahydrofuran and suspended in 300 ml of tetrahydrofuran, cooled to 0°C and 50 g diethyl methylmalonate dissolved in 75 ml of tetrahydrofuran was 15 added dropwise over 45 minutes. After stirring for an additional 30 minutes, 36.8 ml of benzyl chloride was added, followed by 24 ml of tetrahydrofuran. The reaction was then heated to gentle reflux for 3 hours, cooled, poured into 400 ml of water and extracted with ethyl acetate (3 x 500 ml). The combined organic extracts were 20 dried over magnesium sulfate, filtered and concentrated to yield 75 g of diethyl benzylmethylmalonate.

The diethyl benzylmethylmalonate (75 g) was combined 25 with 300 ml of ethanol and a solution of 100 g potassium hydroxide in 300 ml of water and heated to a gentle reflux for 5 hours. After cooling, the mixture was extracted with diethyl ether (2 x 300 ml). The aqueous phase was then acidified with 120 ml concentrated hydrochloric acid, and 30 extracted with diethyl ether (3 x 300 ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated to yield 49.2 g of benzyl-methylmalonic acid as a yellow solid (83% yield).

35 The benzylmethylmalonic acid (49.2 g) was dissolved in 400 ml of acetonitrile with 1.69 g of cuprous oxide and heated to reflux for 5 hours. The solvent was removed under vacuum. The residue was taken up in 400 ml of

diethyl ether and rinsed with 10% hydrochloric acid (2 x 300 ml), 300 ml of saturated sodium chloride, dried over magnesium sulfate, filtered and concentrated. The residue 5 was purified by flash chromatography (5% to 10% methanol in chloroform) to yield 38.3 g of 2-benzylpropionic acid (99% yield).

The 2-benzylpropionic acid (38.3 g) was combined with 10 400 ml of 50% aqueous ethanol, 83.88 g quinine ·2H₂O and heated on a steam bath for 20 minutes to give a clear solution. After standing overnight, the crystals which formed were collected to yield 97.37 g of quinine salt. After six additional recrystallizations from 50% aqueous 15 ethanol, there remained 18.8 g of the quinine salt.

The quinine salt (0.34 g) was treated with 100 ml of 1M sulfuric acid and extracted with chloroform (2 x 100 ml). The combined organic extracts were dried over 20 magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (5% to 10% methanol in chloroform, 2 mm plate) to yield 89 mg of (S)-2-methyl-3-phenylpropionic acid.

25 A 1.0 g quantity of (S)-2-methyl-3-phenylpropionic acid was combined with 0.67 ml of N-methylmorpholine, cooled to -20°C and treated with 0.79 ml of isobutyl chloroformate. After 15 minutes, 1.38 g of 1,3-di-n-propyl-5,6-diaminouracil in 2 ml dimethylformamide was added. The reaction was 30 allowed to warm to room temperature over 2 hours. The reaction was then poured into 300 ml of chloroform, rinsed with 200 ml of saturated sodium bicarbonate, 200 ml of saturated sodium chloride, dried over magnesium sulfate, filtered and concentrated. The residue was purified by 35 radial chromatography (3% to 5% to 10% methanol in chloroform, 2 mm plate) and (10% to 15% isopropyl alcohol in hexane, 2 m plate) to yield 1.6 g of amide. This was purified by flash chromatography (3% to 5% to 10% methanol

-36-

in chloroform) (5% to 10% isopropyl alcohol in hexane) to yield 1.05 g of amide. This was purified by radial chromatography (5% isopropyl alcohol in hexane, 2 mm plate) 5 to yield 0.44 g of amide.

The amide (430 mg) was dissolved in 40 ml of dry benzene and 7.5 ml of triethyloxonium tetrafluoroborate (1M in methylene chloride) was added. The reaction was heated to 10 reflux for 5 hours. It was then poured into phosphate buffer, extracted with 300 ml toluene, dried over magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (3% to 6% methanol in chloroform, 2 mm plate) to yield 209 mg of imino ether and 122 15 mg of starting material. The imino ether was again purified as above to yield 121 mg of pure chiral imino ether.

A 121 mg quantity of the chiral imino ether was dissolved in 14 ml of benzene and heated to reflux for 4 20 hours. Thin layer chromatography indicated complete reaction. The solvent was removed under vacuum and the residue purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 87 mg of material which was recrystallized from 20% diethyl ether in hexane 25 to yield 76 mg of 3,7-dihydro-8-[(S)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione after drying under vacuum at 60°C for 2 hours as a white solid, m.p. 141-142°C.

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EXAMPLE 2

S-(+)-2-Phenylpropionic acid (0.69 g), 0.46 ml of N-methylmorpholine and 10 ml of tetrahydrofuran were combined and cooled to -20°C. An 0.46 ml volume of isobutyl chloroformate was added and the reaction was allowed to stir for 35 25 minutes. An 0.84 g quantity of 1,3-di-n-propyl-5,6-diaminouracil in 5 ml of dimethylformamide was added and the reaction was stirred at -20°C for 4 hours. The solution was then warmed to room temperature overnight. The solvent

-37-

was removed under high vacuum and the residue was taken up in 300 ml of chloroform. The organic layer was rinsed with 200 ml of saturated sodium bicarbonate, 200 ml of saturated 5 sodium chloride, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography (5% to 10% to 15% isopropyl alcohol in hexane) to yield 0.94 g of amide as a foam (69% yield).

10 The above amide (0.90 g) was dissolved in 50 ml of dry benzene, treated with 16.3 ml of triethyloxonium tetrafluoroborate (1M in methylene chloride) and heated to 50°C for 15 hours. The solution was then poured into 300 ml of phosphate buffer and extracted with 400 ml of diethyl 15 ether. The organic phase was rinsed with 300 ml of saturated sodium chloride, dried over magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (3% to 5% to 10% methanol in chloroform, 2mm plate) to yield 0.70 g of imino ether (72% 20 yield).

The above imino ether (0.70 g) was dissolved in 50 ml of dry benzene and heated to reflux under nitrogen for 4 hours. The solvent was removed under high vacuum and the 25 residue was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 0.415 g of product after recrystallization. This was dried under high vacuum over P₂O₅ to yield 0.413 g of product, m.p. 134.5-136°C. This was again recrystallized from 20% diethyl ether in 30 hexane to yield 255 mg of product which was dried under high vacuum in a drying pistol at 39°C for 20 hours to yield 252 mg of 3,7-dihydro-8-[(S)-1-phenylethyl]-1,3-dipropyl-1H-purine-2,,6-dione.

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EXAMPLE 3

N-Valeric acid (1 g) was dissolved in 75 ml of tetrahydrofuran and treated with 2 equivalents of lithium diisopropylamide at room temperature. The solution was then

heated to 40°C for 30 minutes followed by the addition of 1.1 ml of benzyl chloride. After 1.5 hours at 40°C, the reaction mixture was cooled to room temperature, poured 5 into 300 ml of water and extracted with diethyl ether (2 x 200 ml). The aqueous solution was then acidified with 1M hydrochloric acid and extracted with diethyl ether (2 x 300 ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated. The residue 10 was purified by radial chromatography (40-50% ethyl acetate in hexane, 2 mm plate) to yield 1.63 g of 2-benzylpentanoic acid (87% yield).

The 2-benzylpentanoic acid (0.88 g) was dissolved in 15 15 ml of tetrahydrofuran with 0.46 ml of N-methylmorpholine. The solution was cooled to -20°C and 0.60 ml of isobutyl chloroformate was added. After 30 minutes, 0.84 g 1,3-di-n-propyl-5,6-diaminouracil in 5 ml of dimethylformamide was added with stirring at -20°C. After 3 hours, the reaction 20 was warmed to room temperature and the solvent was removed under high vacuum. The residue was purified by flash chromatography (5% to 10% isopropyl alcohol in hexane) to yield 0.55 g of the amide as a foam.

25 The amide (0.55 g) was combined with 20 ml of 30% potassium hydroxide and 5 ml of ethanol and heated to 80°C with stirring for 5 hours. The solution was then cooled, acidified with concentrated hydrochloric acid, extracted with chloroform (3 x 200 ml), and the organic extracts were 30 combined and dried over magnesium sulfate. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate). The product was triturated with 20% diethyl ether in hexane and dried under 35 high vacuum, at 39°C, for 16 hours to yield 217 mg of 3,7-dihydro-8-[1-(phenylmethyl)butyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 158-160°C.

EXAMPLE 4

1,3-Diallyl-6-aminouracil (5 g) was suspended in 400 ml of water in a 1 L round bottom flask with overhead stirring. Acetic acid (6.7 ml of a 20% solution) was added, followed by intermittent addition of 2 ml of concentrated hydrochloric acid and a sodium nitrite solution (1.53 g in 7 ml water). After 4 hours, this solution was filtered, washed with water, collected and dried in a vacuum oven at 80°C for 20 hours to yield 4.54 g of 1,3-diallyl-5-nitroso-6-aminouracil as a purple solid, m.p. 170-180°C (87% yield).

The 1,3-diallyl-5-nitroso-6-aminouracil (4.5 g) was suspended in 150 ml of ethyl acetate and treated with 23.6 g of sodium dithionite in 64 ml of water. After 1 hour, the layers were separated and the aqueous phase was extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated and the residue was purified by flash chromatography (10% methanol in chloroform) to yield 4.41 g of 1,3-diallyl-5,6-diaminouracil.

Next, 2-phenylpropionic acid (1.0 g) was dissolved in 10 ml of acetonitrile with 0.73 ml of N-methylmorpholine at -20°C. Isobutyl chloroformate (0.86 ml) was added. After 15 minutes, 1.48 g of 1,3-diallyl-5,6-diaminouracil in 3 ml of dimethylformamide was added. After 4 hours, the reaction was warmed to room temperature and the solvent was removed under vacuum. The residue was purified twice by flash chromatography (3-5% methanol in chloroform) to yield 0.50 g of the amide.

The amide (0.50 g) was dissolved in 30 ml of dry benzene, treated with 9.2 ml of triethyloxonium tetrafluoroborate (1M in methylene chloride) and heated to 50°C for 5 hours. After cooling, the reaction was poured into phosphate buffer (200 ml) and extracted with toluene (3 x 200

-40-

ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated. The residue was dissolved in 100 ml of toluene and heated to 100°C for 4 hours. After cooling, the solvent was removed under vacuum and the residue was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 0.45 g of a white solid, m.p. 142-143°C. This solid was recrystallized from 30% diethyl ether in hexane to yield 280 mg of 3,7-dihydro-8-(1-phenylethyl)-1,3-di-2-propenyl-1H-purine-2,6-dione.

EXAMPLE 5

Diisopropylamine (3.2 ml) was dissolved in 20 ml of tetrahydrofuran, cooled to 0°C and treated with 14.2 ml of 1.6M *n*-butyllithium. After 30 minutes, the lithium diisopropylamide was added to 1 g of *n*-butyric acid in 75 ml of tetrahydrofuran at -78°C. After 10 minutes the reaction was warmed to -20°C. After 10 more minutes the reaction was warmed slowly to room temperature. The solution was then heated to approximately 35°C for 30 minutes and then cooled back to room temperature and 1.3 ml of benzyl chloride was added. After 1.5 hours the reaction mixture was heated to 35°C for 2.5 hours. The solution was then cooled, diluted with 300 ml of water, rinsed with diethyl ether (2 x 200 ml), and the aqueous phase was acidified with 1M hydrochloric acid and extracted with diethyl ether (3 x 200 ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 1.56 g of 2-benzylbutyric acid (77% yield).

The 2-benzylbutyric acid (0.82 g) was dissolved in 10 ml of tetrahydrofuran, cooled to -20°C, and treated with 0.46 ml of N-methylmorpholine and 0.60 ml of isobutyl chloroformate. After 30 minutes, 0.84 g of 1,3-di-*n*-propyl-5,6-diaminouracil in 5 ml of dimethylformamide was added

-41-

and the reaction mixture was allowed to stir at -20°C for 4 hours. The solution was then allowed to warm to room temperature overnight. The solution was then diluted with 5 200 ml of methylene chloride and rinsed with saturated sodium bicarbonate (100 ml). The organic phase was dried over magnesium sulfate, filtered and concentrated under high vacuum. The residue was purified by flash chromatography (5% to 10% to 15% to 20% isopropyl alcohol in 10 hexane) to yield 1.04 g of amide (71% yield).

The amide (1.04 g) was dissolved in 10 ml of ethanol, followed by the addition of 40 ml of 30% potassium hydroxide and heated to 90°C for 1.5 hours. The solution 15 was then allowed to cool to room temperature overnight, and acidified with concentrated hydrochloric acid. The reaction mixture was diluted with 200 ml water, extracted with chloroform (3 x 200 ml), and the combined organic extracts were dried over magnesium sulfate, filtered and 20 concentrated. The residue was purified by radial chromatography (40-50% ethyl acetate in hexane, 2 mm plate) to yield 0.49 g of product. The product was triturated with 20% diethyl ether in hexane, and the white precipitate was collected and dried at 39°C under high vacuum to yield 418 25 mg of 3,7-dihydro-8-[1-(phenylmethyl)propyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 180°C.

The above product was again dried under high vacuum at 39°C for 6 hours to yield 407 mg of 3,7-dihydro-8-[1-(phenylmethyl)propyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 30 186-187°C. This was dried over anhydrous phosphoric acid at 39°C under high vacuum for 24 hours to yield 342 mg of final product 3,7-dihydro-8-[1-(phenylmethyl)propyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 186-188°C.

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EXAMPLE 6

(R)-(-)-2-Phenylpropionic acid (0.69 g) was combined with 15 ml of tetrahydrofuran, 0.46 ml of N-methylmorpho-

line, and cooled to -20°C and treated with 0.6 ml of isobutyl chloroformate. After 30 minutes, 0.84 g of 1,3-di-n-propyl-5,6-diaminouracil in 5 ml of dimethylformamide 5 was added to the reaction, which was allowed to stir at -30°C for 4 hours. The solution was then allowed to warm to room temperature over 15 hours and the solvent was removed under high vacuum. The residue was taken up in 300 ml of chloroform, and the organic phase was rinsed with 200 10 ml of saturated sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography (5% to 10% to 15% to 20% isopropyl alcohol in hexane) to yield 1.21 g of the desired amide (89% yield).

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The amide (1.1 g) was dissolved in 50 ml of benzene, treated with 19.9 ml of triethyloxonium tetrafluoroborate (1M in methylene chloride) and heated at 50°C for 15 hours. The mixture was then cooled, poured into 300 ml of diethyl 20 ether and rinsed with 200 ml of phosphate buffer, and 200 ml of water, 200 ml of saturated sodium chloride. The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (2% to 5% methanol in chloroform, 2 mm 25 plate) to yield 0.65 g of desired imino ether.

The imino ether (0.65 g) was dissolved in 60 ml of dry benzene and heated at reflux for 4 hours. After cooling, the solvent was removed under vacuum and the residue was 30 purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 0.56 g of 3,7-dihydro-8-[(R)-1-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 136-137°C.

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EXAMPLE 7

2-Phenylpropionic acid (0.69 g) was dissolved in 15 ml of tetrahydrofuran, treated with 0.46 ml of N-methylmorpholine, cooled to -20°C and 0.6 ml of isobutyl chloroformate

was added. After 30 minutes, 0.84 g of 1,3-di-n-propyl-5,6-diaminouracil in 5 ml of dimethylformamide was added. The reaction was allowed to stir at -20°C for 4 hours and 5 then warmed to room temperature. The solvent was removed under high vacuum and the residue was purified by flash chromatography (5% to 10% to 15% to 20% isopropyl alcohol in hexane) to yield 0.96 g of desired amide (70% yield).

10 The amide (0.95 g) was combined with 10 ml of ethanol and 40 ml of 30% of aqueous potassium hydroxide and heated to 90°C for 1.5 hours. The solution was then cooled in an ice bath and carefully acidified with concentrated hydrochloric acid. The reaction mixture was diluted with 100 ml 15 of water and the aqueous layer was extracted with chloroform (3 x 200 ml). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated to yield 0.91 g of product. The product was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm 20 plate) to yield 0.78 g of material which was recrystallized from 20% diethyl ether in hexane to yield 0.591 g of 3,7-dihydro-8-(1-phenylethyl)-1,3-dipropyl-1H-purine-2,6-dione, m.p. 148-150°C.

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EXAMPLE 8

Sodium hydride (15.2g, 50% solution) was rinsed with 100 ml of tetrahydrofuran. It was then suspended in 300 ml of tetrahydrofuran, cooled to 0°C and 50 g of diethyl methylmalonate dissolved in 75 ml of tetrahydrofuran was added 30 dropwise over 45 minutes. After stirring an additional 30 minutes, 36.8 ml of benzyl chloride was added followed by 25 ml of tetrahydrofuran. The reaction mixture was then heated at gentle reflux for 3 hours, cooled, poured into 500 ml of water and extracted with ethyl acetate (3 x 500 ml). The combined organic extracts were dried over 35 magnesium sulfate, filtered and concentrated to yield 75 g of diethyl benzylmethylmalonate.

-44-

The diethyl benzylmethylmalonate (75 g) was combined with 300 ml of ethanol and a solution of 100 g of potassium hydroxide in 300 ml of water and heated at gentle reflux 5 for 5 hours. After cooling, the mixture was extracted with diethyl ether (2 x 300 ml). The aqueous layer was then acidified with 120 ml of concentrated hydrochloric acid and extracted with diethyl ether (3 x 300 ml). The combined organic extracts were dried over magnesium sulfate, 10 filtered and concentrated to yield 49.2 g of benzylmethylmalonic acid as a yellow solid (83% yield).

The benzylmethylmalonic acid (49.2 g) was dissolved in 400 ml of acetonitrile, treated with 1.69 g of cuprous 15 oxide and heated to reflux for 5 hours. The solvent was removed under vacuum and the residue taken up in 400 ml of diethyl ether and rinsed with 10% hydrochloric acid (2 x 300 ml), saturated sodium chloride (300 ml), dried over magnesium sulfate, filtered and concentrated. The residue 20 was purified by flash chromatography (5% to 10% methanol in chloroform) to yield 38.37 g of 2-benzylpropionic acid (99% yield).

The 2-benzylpropionic acid (38.3 g) was combined with 25 400 ml of 50% aqueous ethanol, 83.88 g of quinine ·2H₂O and heated on a steam bath for 20 minutes to give a clear solution. After standing overnight, the crystals which formed were collected to yield 97.37 g of the quinine salt. After six additional recrystallizations from 50% aqueous 30 ethanol there remained 18.8 g of the quinine salt.

The mother liquors from the above recrystallizations were acidified and extracted to yield 24.86 g of recovered 2-benzylpropionic acid. This acid was combined with 18.4 g 35 of d-(+)-α-methylbenzylamine in 160 ml of ethyl acetate, dissolved by heating on a steam bath, cooled, and the precipitate was collected to yield 35 g of the amine salt. After three additional recrystallizations from ethyl

acetate, the amine salt (0.4 g) was treated with 100 ml of 1M sulfuric acid. The aqueous layer was extracted with chloroform (2 x 100 ml) and the combined organic extracts 5 dried over magnesium sulfate, filtered and concentrated. The residue was purified by radial chromatography (5% methanol in chloroform, 2 mm plate) to yield 186 mg of (R)-2-benzylpropionic acid.

10 (R)-2-Benzylpropionic acid (0.69 g) was dissolved in 15 ml of tetrahydrofuran and the solution was cooled to -20°C and treated with 0.46 ml of N-methylmorpholine, 0.60 ml of isobutyl chloroformate and allowed to stir for 30 minutes. This was followed by the addition of 0.84 g of 1,3-di-n-
15 propyl-5,6-diaminouracil in 5 ml of dimethylformamide and the reaction mixture was allowed to stir for an additional 4 hours at -20°C. The solution was allowed to warm to room temperature overnight. The solvent was removed under high vacuum and the purple residue was purified by flash chromatography (5% to 10% to 15% to 20% isopropyl alcohol in
20 hexane) to yield 0.87 g of desired amide (64% yield).

The amide (0.85 g) was dissolved in 100 ml of dry benzene, treated with 14.8 ml of triethyloxonium tetrafluoroborate (1M in methylene chloride) and the solution was 25 heated at 50°C for 15 hours. The solution was then cooled, poured into 500 ml of diethyl ether and rinsed with 300 ml of phosphate buffer, 200 ml of saturated sodium chloride, dried over magnesium sulfate, filtered and concentrated.
30 The residue was purified by radial chromatography (2% to 5% methanol in chloroform, 2 mm plate) to yield 0.36 g of desired imino ether.

The imino ether (0.36 g) was dissolved in 100 ml of dry 35 benzene and heated to reflux for 3 hours. The solvent was removed under vacuum and the residue was purified by radial chromatography (50% ethyl acetate in hexane, 2 mm plate) to yield 0.23 g of 3,7-dihydro-8-[(R)-1-methyl-2-phenylethyl]-

1,3-dipropyl-1H-purine-2,6-dione. This solid was recrystallized from 20% diethyl ether in hexane to yield, after drying under high vacuum at 39°C, 187 mg of 3,7-
5 dihydro-8-[(R)-1-methyl-2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 141-142°C.

EXAMPLE 9

10 β -Propiolactone (5.5 g) was dissolved in 100 ml of methanol and treated with 10.8 ml of triethylamine at room temperature with stirring. After 3 days, the solvent was removed under vacuum and the residue was purified by flash chromatography (10% to 20% isopropyl alcohol in hexane) to yield 3.30 g of 3-hydroxymethyl propionate.

15 The 3-hydroxymethyl propionate (3.23 g) was dissolved in 100 ml tetrahydrofuran, cooled to -50°C and treated with 2.1 eq. of lithium diisopropylamide in 100 ml tetrahydrofuran. After 20 minutes, 3.68 ml of benzyl bromide was
20 added to the dianion at -50°C. The temperature was warmed to -20°C over one hour. The reaction was then diluted with 500 ml of saturated ammonium chloride and the aqueous layer which formed was extracted with diethyl ether (2 x 500 ml). The combined organic extracts were dried over anhydrous
25 magnesium sulfate, filtered, and concentrated. The crude residue was purified by flash chromatography (10% to 20% isopropyl alcohol in hexane) to yield 2.65 g of 2-benzyl-3-hydroxymethyl propionate.

30 The 2-benzyl-3-hydroxymethyl propionate (2.6 g) was dissolved in 75 ml of dry dimethylformamide under nitrogen. t -Butyldimethylsilyl chloride (2.2 g) was added with stirring, followed by the addition of 2.0 g of imidazole. After 1-1/2 hours the reaction was diluted with 500 ml of
35 diethyl ether. The organic phase was rinsed with 50% aqueous sodium chloride (3 x 200 ml), saturated sodium chloride (300 ml), dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was

purified by flash chromatography (5% to 10% isopropyl alcohol in hexane) to yield 3.49 g of methyl 2-benzyl-3-(t-butyldimethylsilyloxy)propionate.

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The methyl 2-benzyl-3-(t-butyldimethylsilyloxy)propionate (3.3 g) was dissolved in 100 ml of methanol, cooled to 0°C and treated with 50 ml of 30% potassium hydroxide with vigorous stirring. The reaction was then allowed to warm to room temperature over five hours. The reaction was diluted with 200 ml of water, rinsed with diethyl ether, and the aqueous solution was cooled to 0°C. Dichloromethane (100 ml) was added, followed by the slow addition of 260 ml of 1M hydrochloric acid, with stirring. The layers were separated and the aqueous layer was extracted with dichloromethane (3 x 200 ml). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by radial chromatography (2% to 4% methyl alcohol in chloroform, 4 mm plate) to yield 2.14 g of the 2-benzyl-3-(t-butyldimethylsilyloxy)propionic acid.

The 2-benzyl-3-(t-butyldimethylsilyloxy)propionic acid (2.1 g) was dissolved in 20 ml of tetrahydrofuran, cooled to -20°C and treated with 0.71 ml of N-methylmorpholine. Isobutyl chloroformate (0.92 ml) was then added and the reaction was allowed to stir for 20 minutes at -20°C. The 1,3-di-*n*-propyl-5,6-diaminouracil (1.62 g) in 10 ml of dimethylformamide was added and the reaction was stirred for 3 hours at -20°C. The reaction was then warmed to room temperature, diluted with 400 ml of chloroform and the organic phase rinsed with 50% aqueous sodium chloride (2 x 200 ml), saturated sodium bicarbonate (200 ml), dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by radial chromatography (5% to 10% methyl alcohol in chloroform, 4 mm plate) to yield 4.25 g of the amide.

The amide (3.1 g) was then dissolved in 50 ml of ethyl alcohol and treated with 100 ml of 30% potassium hydroxide. This was heated to reflux for 1.5 hours. After cooling to 5 0°C, the reaction was acidified with 42 ml of concentrated hydrochloric acid. The aqueous layer was extracted with chloroform (2 x 200 ml). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by 10 radial chromatography (5% to 10% methyl alcohol in chloroform, 4 mm plate) and (5% to 10% to 20% isopropyl alcohol in hexane, 4 mm plate) to yield 1.1 g of crude material. This was triturated with 25% diethyl ether in hexane to yield 0.82 g of 3,7-dihydro-8-[1-(hydroxymethyl)- 15 2-phenylethyl]-1,3-dipropyl-1H-purine-2,6-dione as a white solid, after drying under vacuum at 39°C for 5 hours, m.p. 145-146°C.

EXAMPLE 10

20 Sodium (3.7 g) was dissolved in 80 ml of ethyl alcohol, followed by the addition of 150 ml of diethyl ether. Diethyl malonate (12.5 ml) was added followed by 20 g of α,α'-dibromo-*o*-xylene in 150 ml of diethyl ether with overhead stirring. The reaction was heated to reflux for 5 25 hours. The reaction was cooled, filtered, and the solvent was removed under vacuum. The residue was treated with a potassium hydroxide solution (20 g in 125 ml of water) and heated to reflux for 15 hours. The reaction was then cooled and rinsed with 200 ml of diethyl ether. The 30 aqueous phase was acidified with 30% hydrochloric acid. The precipitate was collected and dried under vacuum over Drierite for 5 hours to yield 8.86 g of indan-2,2-dicarboxylic acid.

35 The indan-2,2-dicarboxylic acid (8.86 g) was placed in a 500 ml, round-bottom flask and heated to 200°C with stirring for 15 minutes. The reaction was then cooled to room temperature and recrystallized from 10% isopropyl

-49-

alcohol in hexane to yield 1.77 g of indan-2-carboxylic acid. (See J. Med. Chem., 23, 1995 (1989).)

5 The indan-2-carboxylic acid (1.0 g) was dissolved in 15 ml of tetrahydrofuran, treated with 0.62 ml of N-methylmorpholine, and cooled to -20°C. Isobutyl chloroformate (0.80 ml) was added and the reaction was stirred for 30 minutes at -20°C. The 1,3-di-n-propyl-5,6-diaminouracil (1.2 g) in
10 5 ml of dimethylformamide was then added and the reaction was stirred at -20°C for 4 hours. After warming to room temperature, the reaction was poured into 300 ml of chloroform and rinsed with 50% aqueous sodium chloride (2 x 100 ml), saturated sodium bicarbonate (2 x 100 ml), dried
15 over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified by radial chromatography (5% to 10% methyl alcohol in chloroform, 4 mm plate) and (10% to 20% to 30% isopropyl alcohol in hexane, 4 mm plate) to yield 2.19 g of the amide.

20 The amide (2.19 g) was treated with 100 ml of 30% potassium hydroxide, 40 ml of ethyl alcohol and heated to reflux for 2 hours. The reaction was then cooled to 0°C and acidified with 42 ml of concentrated hydrochloric acid.
25 The precipitate was collected and dissolved in 300 ml of chloroform. The organic phase was rinsed with 200 ml of saturated sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was triturated with 80% diethyl ether in hexane
30 to yield, after drying under vacuum at 60°C, 1.10 g of 3,7-dihydro-8-(2-indanyl)-1,3-dipropyl-1H-purine-2,5-dione, m.p. 223-224°C.

EXAMPLE 11

35 2-Phenylbutyric acid (1.1 g) was treated with 5,6-diamino-1,3-dipropyluracil to obtain the amide and was cyclized following the procedure in Example 7 to obtain 454

-50-

mg of 3,7-dihydro-8-[(\pm)-phenylpropyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 137-138°C.

5

EXAMPLE 12

(S)-(+)-2-Phenylbutyric acid (0.93 g) was treated with 5,6-diamino-1,3-dipropyluracil to obtain the amide, following the procedure in Example 6. The amide was converted to the imino ether, which was thermally cyclized following the 10 procedure of Example 6 to obtain 547 mg of 3,7-dihydro-8-[(S)-phenylpropyl]-1,3-dipropyl-1H-purine-2,5-dione, m.p. 128-131°C.

EXAMPLE 13

15 (R)-(-)-2-Phenylbutyric acid (0.98 g) was treated with 5,6-diamino-1,3-dipropyluracil to obtain the amide, following the procedure in Example 6. The amide was converted to the imino ether which was thermally cyclized following the 20 procedure of Example 6 to obtain 190 mg of 3,7-dihydro-8-[(R)-phenylpropyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 128-130°C.

EXAMPLE 14

A 1.9 g quantity of 2-benzylpropanoic acid was treated 25 with 0.65 g of potassium hydroxide in 60 ml of water, with stirring. To this solution was added 2.0 g of 5,6-diamino-1,3-dimethyluracil hydrate followed by 2.3 g of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride. After 30 2 hours, the solvent was removed and the residue purified by radial chromatography (40% isopropyl alcohol in hexane, 4 mm plate) to yield 1.85 g of material which was triturated with ether to yield 0.40 g of amide as a white solid.

The amide (0.33 g) was treated with 10 ml of 30% 35 aqueous potassium hydroxide and 2 ml of ethyl alcohol and heated to 70°C for 1.5 hours. After cooling, the reaction was acidified with 55 ml of 1M hydrochloric acid and extracted with 300 ml of ethyl ether. The organic phase

-51-

was rinsed with 200 ml of water, 200 ml of saturated sodium chloride, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was triturated with hexane 5 to yield, after drying under vacuum over phosphorous pentaoxide, 142 mg of 3,7-dihydro-8-[(\pm) -(methyl-2-phenylethyl)]-1,3-dimethyl-1H-purine-2,6-dione, m.p. 198-199°C.

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EXAMPLE 15

A 22 g quantity of 4-benzyloxybenzyl chloride was treated with diethyl methylmalonate anion following the procedure in Example 8. The alkylated methylmalonate was saponified and decarboxylated following the same procedure 15 to obtain 18.06 g of 2-(4-benzyloxybenzyl)propionic acid, m.p. 93-95°C. A 3.6 g quantity of this acid was treated with 5,6-diamino-1,3-dipropyluracil to obtain the amide and was cyclized following the procedure in Example 7 to obtain 1.46 g of material which was recrystallized from 5% ethyl 20 ether in hexane to yield 0.72 g of 3,7-dihydro-8-[methyl-2-(4-benzyloxyphenyl)ethyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 124-126°C. A 260 mg quantity of 3,7-dihydro-8-[methyl-2-(4-benzyloxyphenyl)ethyl]-1,3-dipropyl-1H-purine-2,6-dione was dissolved in 20 ml of methyl alcohol and 25 treated with a catalytic amount of 5% palladium on charcoal. This was placed under an atmosphere of hydrogen for 2 hours with stirring. It was then filtered through Celite and the filtrate concentrated under vacuum. The residue was purified by radial chromatography (50% ethyl 30 acetate in hexane, 4 mm plate) to yield 182 mg of material which was triturated with 5% ethyl ether in hexane to yield, after drying under high vacuum at 39°C for 3 hours, 162 mg of 3,7-dihydro-8-[methyl-2-(4-hydroxyphenyl)ethyl]-1,3-dipropyl-1H-purine-2,6-dione, m.p. 218-220°C.

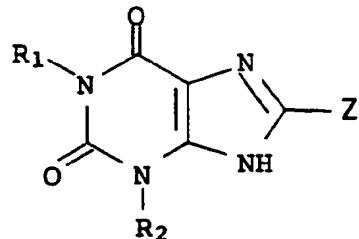
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-52-

WHAT IS CLAIMED IS:

1. A method of attenuating a cognitive deficit in a
 5 patient in need thereof comprising administering to the
 patient a compound according to the structure:

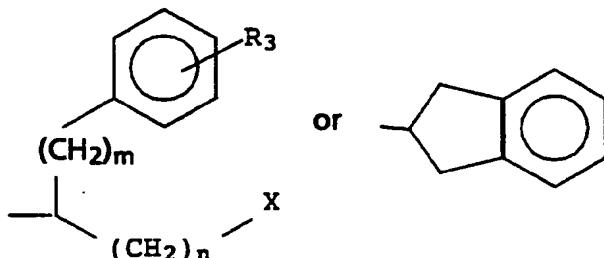
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including the (R) and (S) enantiomers and racemic mixtures thereof, and the pharmaceutically acceptable salts thereof, wherein R₁ and R₂ are each independently (C₁-C₄)lower alkyl or (C₂-C₄)lower alkenyl, Z is:

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R₃ is (C₁-C₃)lower alkyl, nitro, amino, hydroxy, fluoro, bromo or chloro, m is zero or an integer from 1 to 4, n is an integer from 1 to 4, and X is H or OH.

30

2. A method according to claim 1 wherein the compound is 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-1*H*-purine-2,6-dione.

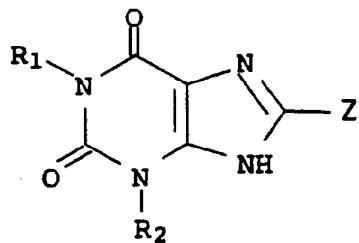
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3. A method according to claim 1 wherein the compound is (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine .

-53-

4. A method of enhancing cognition in a patient in need thereof comprising administering to the patient a compound according to the structure:

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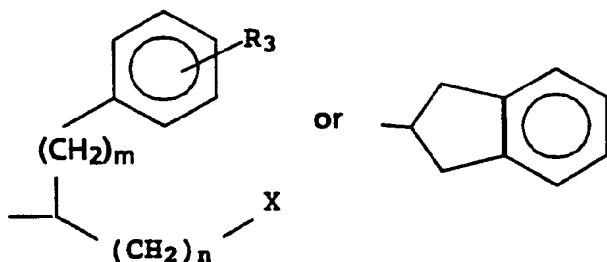


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including the (R) and (S) enantiomers and racemic mixtures thereof, and the pharmaceutically acceptable salts thereof, wherein R₁ and R₂ are each independently (C₁-C₄)lower alkyl or (C₂-C₄)lower alkenyl, Z is:

15

20



R₃ is (C₁-C₃)lower alkyl, nitro, amino, hydroxy, fluoro, bromo or chloro, m is zero or an integer from 1 to 4, n is an integer from 1 to 4, and X is H or OH.

5. A method according to claim 4 wherein the compound is 3,7-dihydro-8[(R)-1-methyl-2-phenylethyl]1,3-dipropyl-30 1H-purine-2,6-dione.

6. A method according to claim 4 wherein the compound is (R)-1,3-dipropyl-8-(1-phenylpropyl)-xanthine.

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FIG. I

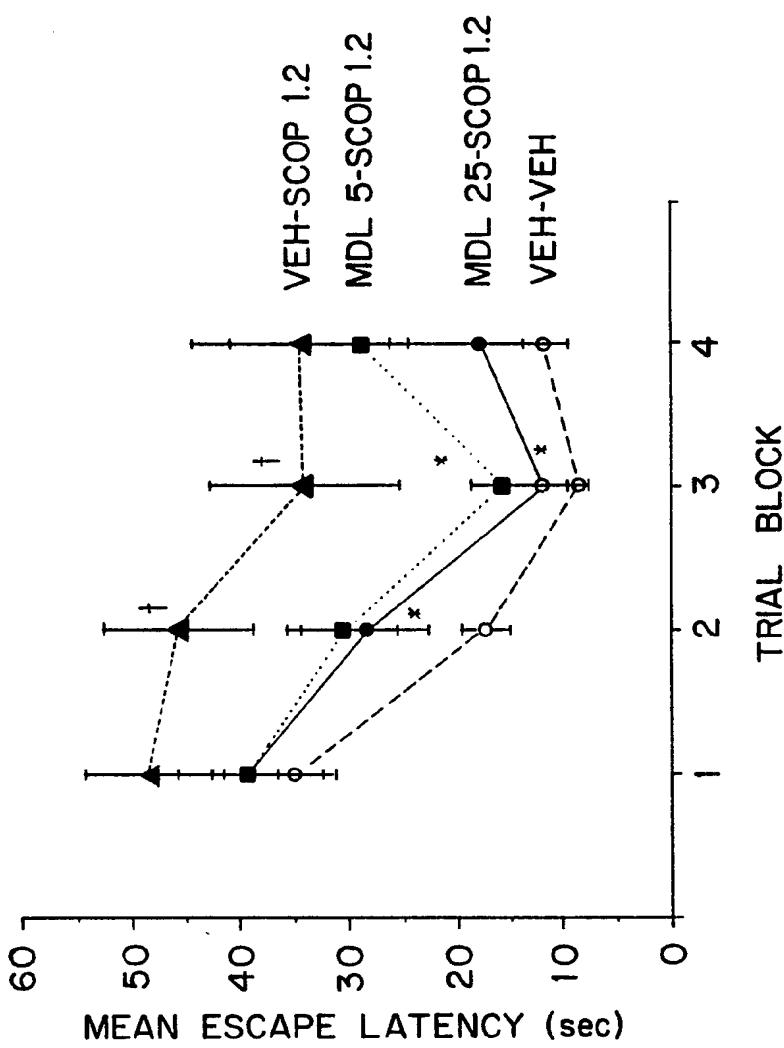


FIG.2

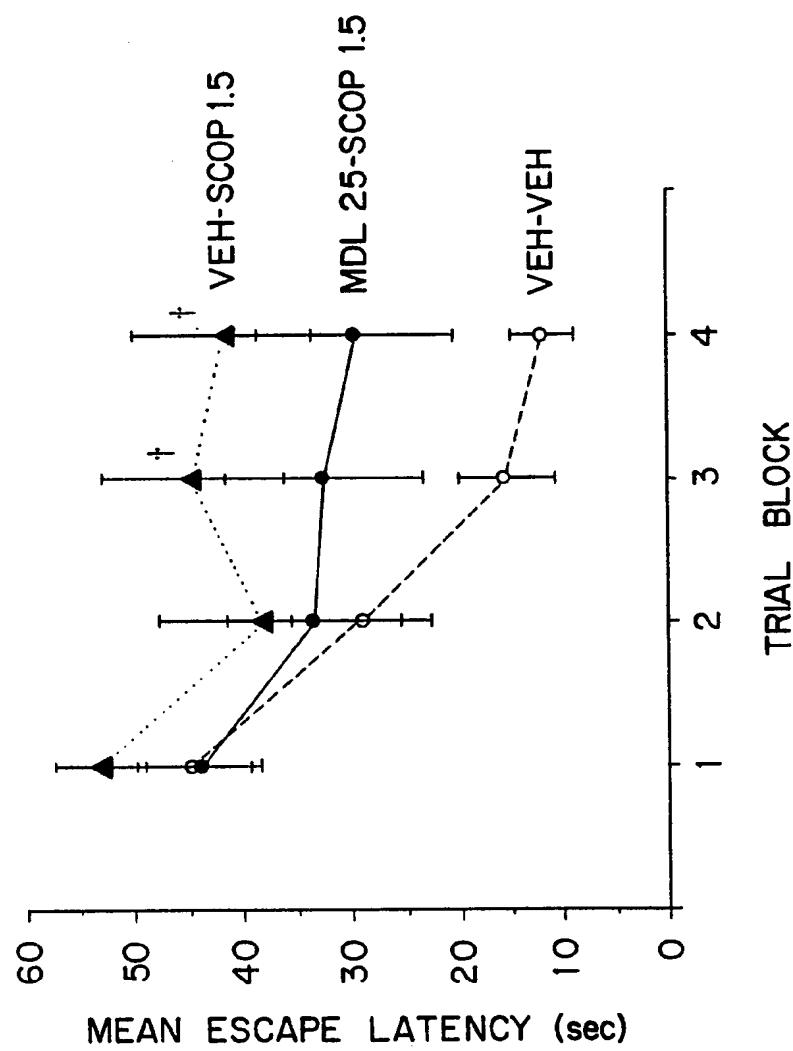
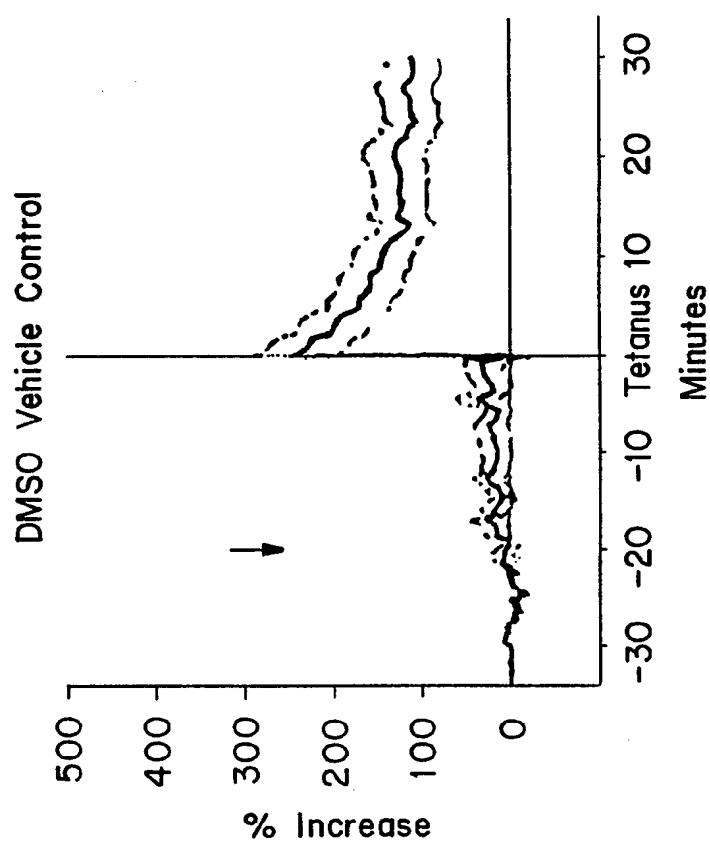


FIG. 3a

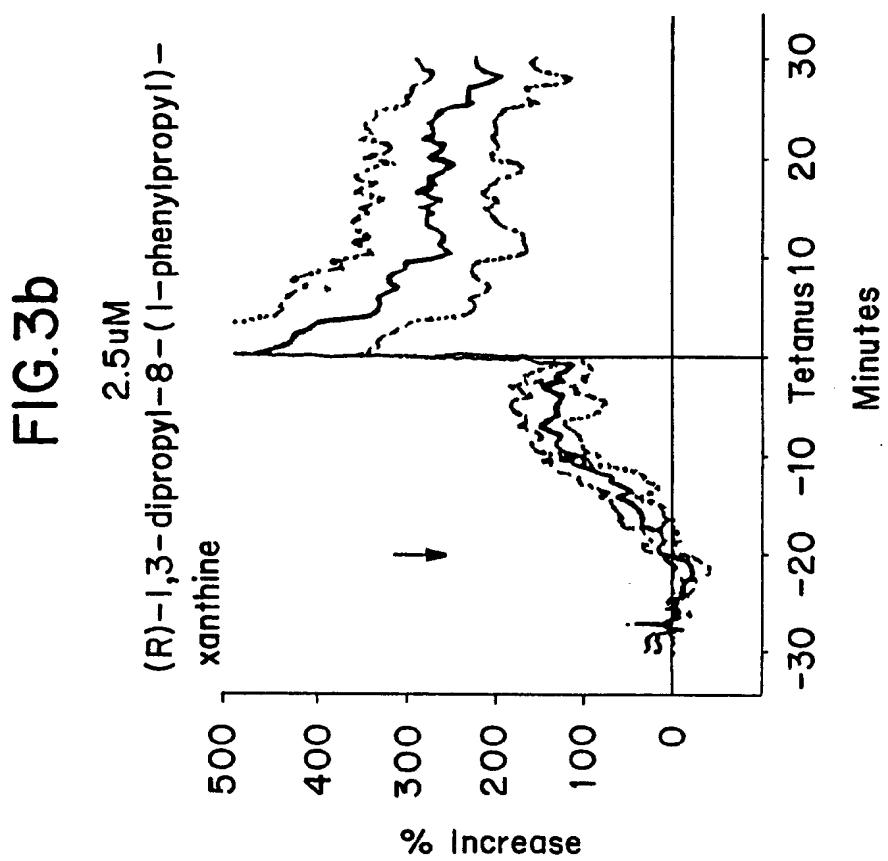
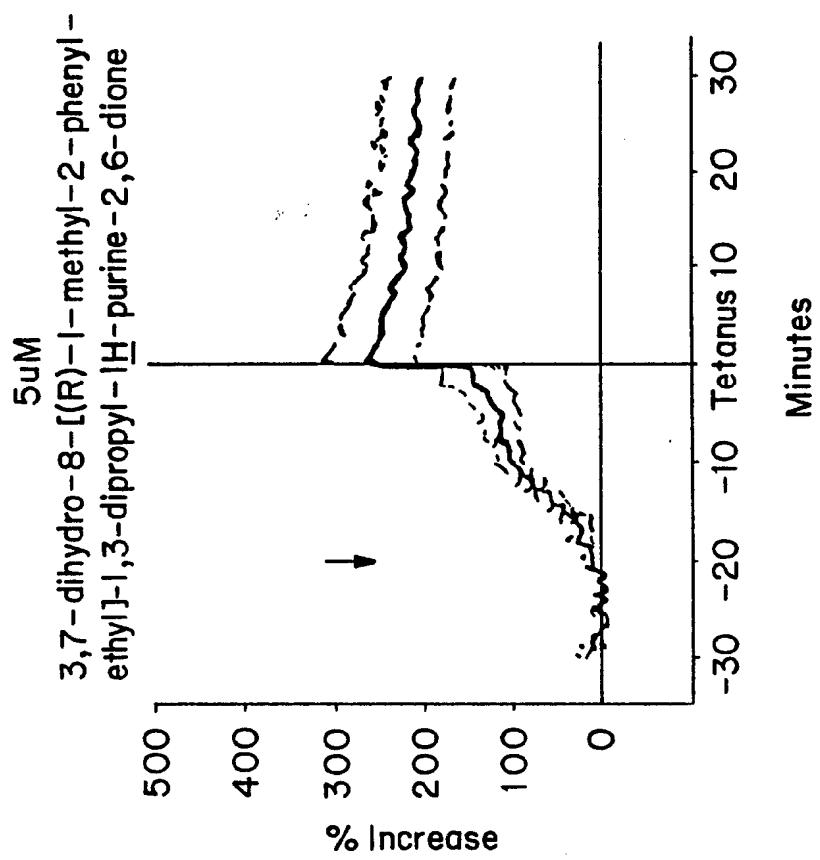


FIG. 3C



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/01009

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 5 C07D473/06 C07D473/08 A61K31/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 5 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| X | EP,A,0 449 175 (MERRELL DOW PHARMACEUTICAL INC.) 2 October 1991 *Document* --- | 1-6 |
| X | JOURNAL OF MEDICINAL CHEMISTRY vol. 35, no. 5 , 6 March 1992 , WASHINGTON pages 924 - 930 JUNICHI SHIMADA ET.AL. *Page 924* *Page 925: table 2, compound 18* --- | 1-6 |
| X | JOURNAL OF MEDICINAL CHEMISTRY vol. 34, no. 1 , January 1991 , WASHINGTON pages 466 - 469 JUNICHI SHIMADA ET. AL. *Page 467: table 467: compound 12 and 15 --- -/-- | 1-6 |

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Date of the actual completion of the international search

25 July 1994

Date of mailing of the international search report

29.07.94

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Luyten, H

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| X | JOURNAL OF MEDICINAL CHEMISTRY vol. 33, no. 12 , 1990 , WASHINGTON pages 3127 - 3130 PEET NORTON P. ET. AL. *Article* --- | 1-6 |
| A | EP,A,0 503 563 (MERRELL DOW PHARMACEUTICALS INC.) 16 September 1992 *Page 33-35: claims* *Page 1-2* --- | 1-6 |
| A | WO,A,92 00297 (BOEHRINGER INGELHEIM INTERNATIONAL) 9 January 1992 *Page 65-78: claims* --- | 1-6 |
| A | EP,A,0 374 808 (BOEHRINGER INGELHEIM) 27 June 1990 *Page 30-39: claims* ----- | 1-6 |

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 94/01009

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|--|------------------|-------------------------|---------|------------------|
| EP-A-0449175 | 02-10-91 | US-A- | 5047534 | 10-09-91 |
| | | AU-B- | 632914 | 14-01-93 |
| | | AU-A- | 7353791 | 03-10-91 |
| | | CN-A- | 1055181 | 09-10-91 |
| | | HU-B- | 208824 | 28-01-94 |
| | | JP-A- | 4221384 | 11-08-92 |
| EP-A-0503563 | 16-09-92 | US-A- | 5208240 | 04-05-93 |
| | | AU-B- | 643599 | 18-11-93 |
| | | AU-A- | 1213892 | 17-09-92 |
| | | JP-A- | 5112568 | 07-05-93 |
| | | NZ-A- | 241890 | 27-04-94 |
| WO-A-9200297 | 09-01-92 | DE-A- | 4019892 | 02-01-92 |
| | | CA-A- | 2064742 | 23-12-91 |
| | | EP-A- | 0487673 | 03-06-92 |
| | | JP-T- | 5501265 | 11-03-93 |
| EP-A-0374808 | 27-06-90 | DE-A- | 3843117 | 28-06-90 |
| | | AU-B- | 637990 | 17-06-93 |
| | | AU-A- | 4707289 | 28-06-90 |
| | | CA-A- | 2006387 | 22-06-90 |
| | | DE-U- | 8817122 | 04-02-93 |
| | | NZ-A- | 231901 | 23-12-93 |
| | | PL-B- | 162877 | 31-01-94 |
| | | US-A- | 5175291 | 29-12-92 |